EFFECTS OF SWIMMING AND ACCESS TO SWEET FOOD ON NICOTINE CESSATION-INDUCED WEIGHT GAIN

1990

POPP

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Abstract

Title of Dissertation: Effects of Swimming and Access to Sweet Food on Nicotine Cessation-induced Weight Gain

Kathryn Anne Popp, Doctor of Philosophy, 1990

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One reason given by cigarette smokers for continuing to smoke despite the serious health consequences of cigarette smoking is that they do not want to gain weight by quitting smoking. Although the inverse relationship between nicotine and body weight is well-established, effective methods for preventing nicotine cessation-induced weight gain have not been determined. Exercise and restricting access to sweet foods may prevent this weight gain. This study used a chronic nicotine administration paradigm in rats, which has previously provided findings concerning nicotine and body weight that generalize to human smokers, to examine the effects of exercise and access to sweet food on nicotine cessation-induced weight gain, food intake, energy efficiency, plasma insulin levels, and on an index of fatness. Nicotine administration reduced rate of weight gain, caloric intake, sweet food intake, and energy efficiency. Nicotine cessation increased rate of weight gain, total caloric intake, sweet food intake, and energy efficiency compared to nicotine

administration levels. Proportion of body fat remained lower in nicotine-treated rats than in saline controls five weeks after cessation of nicotine administration. Exercise reduced rate of weight gain, total caloric consumption, energy efficiency, proportion of body fat, and plasma insulin levels. Access to sweet food reduced rate of weight gain, caloric consumption, and food efficiency in rats receiving nicotine, but not in saline controls. Access to sweet food also reduced caloric consumption during nicotine cessation, but increased proportion of body fat and plasma insulin levels.

The results of this study indicate that exercise provides effective protection against nicotine cessation-induced weight gain, regardless of access to sweet food. However, restricting access to sweet food reduces proportion of body fat in sedentary rats. The results of this study also indicate that changes in energy efficiency, in addition to changes in food intake, underlie the inverse relationship between nicotine and body weight. These findings suggest that cigarette smokers who exercise after quitting smoking will not have to avoid sweet foods in order to prevent weight gain. In contrast, sedentary ex-smokers should avoid sweet foods, in particular, to prevent increases in body fat.

EFFECTS OF SWIMMING AND ACCESS TO SWEET FOOD ON NICOTINE CESSATION-INDUCED WEIGHT GAIN

by

Kathryn A. Popp

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INTRODUCTION

Although smoking causes various types of cancer, heart disease, chronic obstructive lung disease, as well as other health problems, and contributes to more than 390,000 deaths per year in the United States (USDHHS, 1989), approximately 29% of adults in the United States smoked in 1987 (USDHHS, 1989). Research has focused on the reasons people smoke in spite of the well-established health consequences. One of the effects of tobacco use that contributes to the initiation and maintenance of smoking behavior is the control of body weight (USDHHS, 1988). In fact, some smokers report that they smoke, or do not quit smoking, in order to control their body weight (Charlton, 1984; Klesges & Klesges, 1988). Therefore, research concerning the prevention or treatment of weight gain after quitting smoking may enable smokers to quit smoking without gaining unwanted weight.

The inverse relationship between smoking and body weight is supported by both cross-sectional and longitudinal studies. A review (USDHHS, 1988) of cross-sectional studies of the relationship between smoking and body weight found that 89% of studies between 1970 and 1987 reported that smokers weigh less than nonsmokers. Eighty-six percent of the longitudinal studies reviewed supported the inverse relationship between smoking and body weight. In general, smokers who quit smoking gained weight, and this weight gain was greater than the weight gain of nonsmokers (USDHHS, 1988).

Nicotine, an addictive component of tobacco, produces changes in body weight that are similar to those found in smokers (USDHHS, 1989). In animals, nicotine administration causes reductions in body weight, or in rate of weight gain, and cessation of nicotine administration leads to increases in rate of weight gain (Bowen, Eury, & Grunberg, 1986; Grunberg, 1982, 1985, 1986; Grunberg, Bowen, & Morse, 1984; Grunberg, Bowen, & Winders, 1986; Grunberg, Winders, & Popp, 1987; McNair & Bryson, 1983; Schechter & Cook, 1976; Winders & Grunberg, 1989). Studies of smokers obtaining nicotine by chewing nicotine polacrilex gum while quitting smoking also support the inverse relationship between nicotine and body weight (Emont & Cummings, 1987; Fagerstrom, 1987; Gross, Stitzer, & Maldonado, 1989). In both studies, ex-smokers who chewed greater amounts of the gum gained less weight than those chewing lesser amounts, and this inverse relationship was particularly strong for heavy smokers. Taken together, the research literature indicates that nicotine administration and cessation cause changes in body weight, and that animal models yield results regarding nicotine and body weight that are similar to the effects of cigarette smoking on body weight in humans.

Although extensive research has established the inverse relationship between smoking, or nicotine administration, and body weight, the optimal method to prevent weight gain during nicotine cessation remains to be determined. The present study used an animal model to

determine whether manipulation of physical exercise and availability of specific foods prevents weight gain during nicotine cessation. Research concerning behavioral and biological factors involved in the relationship between nicotine and body weight is reviewed. This summary indicates the potential value of manipulating physical activity and specific food availability to control body weight gains during nicotine cessation. Next, behavioral and biological effects of exercise that may be relevant to its role in weight gain following nicotine cessation are discussed. Then, the present study, which was designed to examine the effects of physical activity and specific food availability on post-nicotine weight gains, is presented.

Nicotine, Body Weight, and Energy Intake

The inverse relationship between nicotine and body weight results from changes in energy intake and energy expenditure. This section summarizes evidence from human and animal studies indicating that nicotine administration and cessation alters consumption of foods, especially sweet foods.

Human Studies. Several human studies have demonstrated an increased caloric consumption associated with smoking cessation (Hatsukami, Hughes, Pickens, & Svikis, 1984; Robinson & York, 1986; Stamford, Matter, Fell, & Papenek, 1986). In a cross-sectional laboratory investigation, Grunberg (1982) found that smokers who were allowed to smoke ate less of the sweet foods than nonsmokers or smokers who were not smoking. Consumption of the non-sweet foods (salty

or bland) did not differ according to smoking status of the subjects. Also, smokers tended to select non-sweet foods to eat again in another part of the experiment. Grunberg (1982) concluded that smoking resulted in a decreased preference for and consumption of sweet foods in particular. In a longitudinal study, Rodin (1987) found that smokers who gained weight after quitting smoking increased their sugar consumption. In general, the human studies support the conclusion that the inverse relationship between smoking and body weight results, at least partially, from the differences in food consumption, especially sweet food consumption, that occur with changes in smoking behavior.

Therefore, preventing an increased consumption of sweet food should reduce weight gain following nicotine cessation. One way to do this would be to restrict availability of sweet foods. Based on the research literature, weight gain during nicotine cessation should be attenuated if no sweet foods are available.

Animal Studies. Animal experiments also indicate that nicotine alters eating behavior in ways that are consistent with the weight changes resulting from its administration and cessation. Specifically, nicotine administration is accompanied by decreased food intake and cessation of nicotine is accompanied by increased food intake. There are two general approaches that have been used in animal studies of nicotine and body weight. In one, nicotine is administered acutely, usually by injection or smoke exposure, a few times

per day for many days. In the other, nicotine is administered continuously for many days.

The acute administration studies usually only examine the effects of nicotine administration on body weight and food intake, and do not examine the effects of nicotine cessation. In general, these studies report that nicotine decreases food intake and body weight (Becker & King, 1966; Hughes, Jones, & Nicholas, 1970; Passey, Elson, & Lewis, 1959; Winders & Grunberg, 1989).

In animal studies that used continuous nicotine administration via osmotic minipumps for 2-3 weeks, food and body weight results during both administration and cessation were consistent with those of human studies of smoking and eating (Grunberg, 1982; Rodin, 1987). Grunberg (1982) reported that rats receiving nicotine gained less weight than the control rats during drug administration, and gained weight at a faster rate than control rats during drug cessation. Consumption of bland laboratory chow was similar for all groups throughout the study, but consumption of sugar solutions differed among the groups. Nicotine administration decreased consumption of the sugar solutions, and nicotine cessation increased consumption of the sugar solutions. The changes in sugar solution consumption were consistent with the changes in body weight (Grunberg, 1982). In another experiment, eating behavior and body weight data for rats with access to ground Oreo cookies, potato chips, and laboratory chow followed a similar pattern of results. Rats receiving

nicotine ate less of the ground Oreo cookies than control rats and gained less weight. During nicotine cessation, rats that had received nicotine ate more Oreo cookies and gained weight at a faster rate than control rats (Grunberg, Popp, & Winders, 1988).

In order to determine if sweet taste or carbohydrate content of food were factors involved in nicotine's effects on eating behavior, Grunberg and co-workers (1985) ran two rat experiments. One experiment provided bland laboratory chow and a saccharin-sweetened form of the chow. The other experiment provided bland laboratory chow and a nonsweet high-carbohydrate form of the chow with maltose dextrin added. In both studies, nicotine administration resulted in lower body weights, and nicotine cessation led to increased rates of weight gain. Consumption of the sweet food decreased during nicotine administration and increased during nicotine cessation. The high-carbohydrate food produced a similar, but less pronounced pattern of results. Based on these experiments, both sweet taste and carbohydrate content are important factors in nicotine's effects on food consumption, but sweet taste is particularly important.

Grunberg, Bowen, and Morse (1984) used the same paradigm but provided only bland laboratory chow and water. Nicotine administration and cessation did not affect food consumption in male rats. Nicotine's effects on body weight were similar but reduced compared to changes in weight occurring when sweet food was available and food intake

changed, indicating that alterations in food intake contribute to the inverse relationship between nicotine and body weight. Because nicotine affected body weight in the absence of alterations in food consumption, mechanisms other than changes in energy intake must be involved in nicotine's inverse relationship to body weight. This conclusion is consistent with results from some acute animal experiments reporting changes in body weight in response to nicotine without accompanying changes in laboratory chow intake (Evans, Hughes, & Jones, 1967).

Availability and consumption of sweet foods contributes to weight changes accompanying nicotine administration and cessation. However, the animal research literature indicates that energy expenditure also contributes to these weight changes.

Nicotine, Body Weight, and Energy Expenditure

The inverse relationship between nicotine and body weight is mediated by changes in energy expenditure as well as by changes in energy intake. Energy expenditure involves both behavioral and biological factors. Behavioral factors involve physical activity, and biological factors include changes in resting metabolic rate and thermogenesis.

Research concerning the role of physical activity in the relationship between nicotine and body weight includes studies of animals and humans. Grunberg and Bowen (1985) used minipumps to administer nicotine chronically and found an increase in physical activity in male rats receiving nicotine.

However, this increase in activity occurred after the decrease in weight gain and accounted for less than one percent of the variance in body weight compared to the fifteen percent that nicotine contributed. During nicotine cessation, rats that had received nicotine were less active than control rats. This change in physical activity level accounted for only a small portion of the variance in body weight, but may have contributed to the cessation weight gain. Bowen, Eury, and Grunberg (1986) used the same paradigm, but studied female rats and found no consistent changes in physical activity in response to chronic nicotine administration and cessation.

Other animal experiments concerning nicotine administration and physical activity have been performed, however these experiments were acute both in pattern of nicotine administration and time period in which physical activity was measured. Because chronic nicotine administration may produce tolerance, the applicability of acute studies to human smokers is questionable. The reported effects of acute nicotine administration on physical activity were inconsistent, with variables such as time of day of administration (Bovet et al., 1967), dose injected (Morrison, 1969; Fleming & Broadhurst, 1975), apparatus used to assess physical activity (Bryson, Biner, McNair, Bergondy, & Abrams, 1981), gender, and genotype (Hatchell & Collins, 1980) influencing nicotine's effects on physical activity. Bryson and co-workers (1981) found that physical activity increased with increasing dosage of nicotine in a photocell chamber.

In contrast, physical activity measured in an activity wheel initially decreased, then increased slightly, in response to the low dosage of nicotine. They also reported sex x dose and age x dose interactions for the photocell chamber, but not for the activity wheel. In addition, Hatchell and Collins (1980) reported that nicotine administration depressed motor activity, and that gender influenced activity levels, brain nicotine levels, and liver nicotine levels. They also reported that strain influenced brain and liver nicotine levels. However, neither brain nor liver nicotine levels were related to physical activity. In addition, none of these acute studies looked at physical activity during nicotine cessation.

In light of the inconsistency in and the multitude of factors that influence nicotine's effects on physical activity, it is unlikely that nicotine administration alters body weight through its effects on physical activity. Consistent with this interpretation, several human studies have found no effect of smoking cessation on physical activity (Hall, Ginsberg, & Jones, 1986; Hatsukami et al., 1984; Rodin, 1987; Stamford et al., 1986).

Overall, it appears that changes in physical activity that occur in response to nicotine administration or cessation play a small role in the inverse relationship between nicotine and body weight. This conclusion, however, does not rule out the possibility that physical exercise might be used to prevent or attenuate the weight gains after cessation of

nicotine. This possibility has never been explored empirically prior to this study.

Exercise and Body Weight

Exercise is a component of many weight-loss programs and, therefore, also may prevent weight gain during nicotine cessation. The exercise and body weight literature is extant and has been comprehensively reviewed (Geliebter, 1982; Stern, 1983). Research concerning the contributions exercise makes to weight loss has focused on both energy intake and energy expenditure effects of exercise. Both types of mechanisms will be discussed after the more general issue of exercise effects on body weight.

Generally, exercise reduces weight in obese humans and animals, but does not substantially reduce body weight of lean humans and animals. Boileau, et al. (1971) found that obese students lost an average of 7 pounds during a nine-week exercise program, while lean students lost only 2.2 pounds. Food intake was unregulated in this study. Another study using human subjects exercised the subjects without manipulating diet and resulted in significant weight loss for a group of sedentary obese men (Leon, Conrad, Hunninghake, & Serfass, 1979). During the 16-week exercise program the men's body weight decreased an average of 12.5 pounds. Roughly 13 pounds of fat tissue were lost and 0.5 pounds of lean body mass was gained. Apparently, exercise alone produces weight loss in humans, and this weight loss is primarily due to changes in body fat.

The findings of animal studies generally parallel those of human studies. Hoffman-Goetz and MacDonald (1983) found that treadmill exercise reduced body weight in diet-induced obese male rats, but not in non-obese male rats. Unfortunately, the investigators were not able to induce obesity by dietary manipulation in their female rats, but exercise did not reduce body weight in any of the female rats. However, Hill, Davis, and Tagliaferro (1983) found that female rats that swam gained less weight when provided with an obesity-inducing "cafeteria diet" (palatable foods blended in sweetened, condensed milk) than sedentary female rats given the same diet. Rats in the sedentary-cafeteria diet group weighed 384 g, compared to those in the exercised-cafeteria group (295 g), the sedentary-laboratory chow group (319 g), and the exercised-laboratory chow group (289 g). Even ad libitum wheel running, rather than forced exercise, prevents obesity induced by a high-fat diet (Pitts, 1984). As with Hill et al.'s (1983) female rats, the differences between the exercising and sedentary groups in the Pitts (1984) experiment were strikingly large. Rats eating laboratory chow and living in standard cages had 119 g of body fat, compared to 303 g of body fat in rats with the same housing conditions that ate a high-fat diet. Rats eating a high-fat diet, but having access to a running wheel, had 114 g of body fat. Similar to the findings of these experiments with rats, Mayer et al. (1954) found that exercise reduced body weight in obese male and female mice, but did not affect weight gain in non-obese mice.

A few reports in the animal literature indicate that exercise may reduce body weight in lean rats as well (Mayer et al., 1954). Stevenson and co-workers (1966) found that swimming reduced rate of weight gain in non-obese rats. Sedentary rats gained 46 g during the 4-week swimming period, while rats that swam 1 hour/day, 4 days per week gained 22 g. Rats that swam 2 and 4 hours per day gained 0 g and 3 g, respectively. One possible reason for the reduction in weight gain of these exercising lean rats is the probable increase in energy expenditure for thermoregulation. These swimming rats were spending long periods of time in water that was 30 degrees C and the rats had colonic temperatures between 31.8 and 33.3 degrees C at the end of swimming periods. In another experiment where exercise produced weight loss or reduction in weight gain in lean rats, Mayer et al. (1954) found that non-obese rats running on a treadmill for periods of time up to an hour per day lost weight, as did those that spent 6 hours or more per day on the treadmill. However, treadmill exercise lasting between 1 and 6 hours per day did not affect their body weight. The 6 or more hours of exercise per day phase was referred to as a period of "exhaustion." It seems that daily duration of exercise influences weight loss, with exercise of moderate duration tending to reduce body weight, even in lean animals.

Mayer, Roy, and Mitra (1956) performed a correlational human study with similar results to the animal study. The relationship between physical activity level, food intake, and

body weight was studied in male subjects in West Bengal, India. The sample included men between 5'2" and 5'4" tall. Sedentary men were heavier than men in the light, medium, heavy, and very heavy work categories, and increasing levels of activity from sedentary to light work categories were associated with lower body weights. The mean body weight remained constant for all groups engaging in greater levels of activity than the light work category. Thus, light to moderate increases in levels of physical activity are likely to be most effective in weight loss programs.

Further support for the use of light or moderate exercise levels for weight control programs is provided by the animal study by Pitts (1984). The exercise used in this study was ad libitum rather than forced and, as a result, was probably relatively light exercise.

Overall, it appears that exercise reduces weight in obese humans and animals. This generalization applies to studies that used genetically obese individuals and to studies that examined obesity induced by availability of high-caloric foods. Therefore, it seems reasonable to postulate that exercise would prevent or attenuate excessive weight gain during nicotine cessation that results from increased consumption of sweet, high-caloric foods. In addition, there is evidence from the animal literature that swimming per se decreases body weight even in lean animals. Therefore, swimming may be a particularly effective exercise to avoid weight gains during nicotine cessation. In fact, in a pilot

study for the proposed work, swimming for 30 min/day, 6 days/week for 5 weeks resulted in body weights that were 13% lower than body weights of controls in male Sprague-Dawley rats.

Exercise and Food Intake

Research on exercise and food intake also is extant and has been reviewed (Stern 1983; Pi-Sunyer, 1987; Geliebter, 1982; Oscai, 1973). Most of this research has been done with animals because of the difficulty of measuring long-term food consumption in humans. Generally, human studies measuring food intake in response to exercise show that lean individuals increase food intake and maintain body weight (Woo & Pi-Sunyer, 1985), while obese people either maintain or slightly decrease their food intake and lose weight (Woo, Garrow, & Pi-Sunyer, 1982; Leon et al., 1979).

Reviews of literature on exercise and food intake in animals conclude that male and female animals respond differently to exercise. Male animals usually reduce food intake in response to exercise, while female animals maintain or increase their food intake (Oscai, 1973; Pi-Sunyer, 1987; Stern, 1983). Most of the animal literature is based on studies of lean, rather than obese rats. In a study using both obese and lean rats, Hoffman-Goetz and MacDonald (1983) found that obese male rats did not change their food consumption when exercised. However, in this experiment obesity was induced in these rats by providing a high-fat diet. Therefore, the maintenance of the pre-exercise level

of food intake may have resulted from the fact that the food was highly palatable, rather than from the fact that the animals were obese.

As with body weight, intensity of exercise or total exercise load modifies the relationship between food intake and exercise. Mayer et al. (1956) found that men with jobs classified as sedentary had higher caloric intakes than men whose occupations were classified as "light work." As work level increased from light to heavy, food intake also increased. It appears that relatively light levels of exercise may be most effective in reducing food intake. In support of this generalization, Swenson and Conlee (1979) estimated changes in caloric intake of men in a low or a high intensity exercise group (based on energy expenditure during exercise and body composition changes) and concluded that the low intensity exercise group decreased food consumption by 8 kcal/day and the high intensity exercise group increased consumption by 179 kcal/day.

Mayer et al. (1954) found a similar relationship between exercise and food intake in female rats. Food intake decreased with exercise on the treadmill for up to an hour. One to six hours per day of treadmill running increased food intake and 6 hours or more on the treadmill reduced food intake. Also, Stevenson, Box, Feleki, and Beaton (1966) found that up to two hours of swimming per day reduced food intake in male rats. Four hours of swimming per day did not reduce food intake. In contrast, Katch, Martin, and Martin (1979)

found that high intensity exercise that was equal in caloric expenditure to the low intensity exercise reduced food consumption per gram of body weight compared to the low group. However, this study was only conducted for two 1-week periods with one sedentary week in between the exercise weeks. As a result of the design of the experiment, only relatively short-term effects of exercise intensity were observed.

Short-term effects of exercise include a reduction of food intake, while long-term exercise may increase food intake. Rats eat less on exercise days than on rest days (Thomas & Miller, 1958; Stevenson et al., 1966). Longer periods of exercise are associated with increased food intake, but long-term exercise may also be associated with a heavier exercise load than short-term exercise. The heavier load of exercise may increase food intake, rather than the long-term nature of the exercise. Men and women runners (averaging between 55 and 65 km/week) consumed more calories than nonrunning controls (Blair et al., 1981). The runners were running substantial distances, which might correspond to heavier work loads, and consequently higher food intakes, in Mayer's studies.

Literature on the effects of exercise on food intake in both humans and animals indicates that exercise contributes to weight loss or reduction of weight gain through changes in both food intake and energy expenditure. Exercise reduces weight in obese humans when ad libitum food intake is reduced (Leon et al., 1979) and reduces weight even when there is no

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change in food intake (Woo, Garrow, & Pi-Sunyer, 1982). Also, in lean and obese male rats exercise reduces the rate of weight gain, while it decreases food intake in lean rats and does not change food intake in obese rats (Hoffman-Goetz & MacDonald, 1983).

Based on the studies by Mayer and co-workers (1954, 1956) and Stevenson and co-workers (1966), relatively light levels of exercise may be most effective in reducing body weight and food intake. Also, because food intake is reduced on exercise days, frequent exercise would probably be more effective than infrequent exercise in reducing food intake and body weight during smoking cessation.

Exercise and Energy Expenditure

Dietary changes rather than physical activity changes have often been the focus of individuals who want to lose weight. One possible reason for the lack of interest in exercise as a tool for weight loss is the belief that the energy expenditure of the exercise would be counteracted by increased food consumption. However, the studies that have reported an increase in food intake in response to exercise generally used lean subjects and/or relatively high levels of exercise.

Another reason people may not use exercise to lose weight is the relatively small caloric cost of the exercise itself. However, exercise appears to contribute to weight loss through increasing energy expenditure in at least three ways in addition to the caloric cost of the exercise.

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Exercise increases metabolic rate and this elevation may be maintained for an extended period of time. For example, Edwards, Thorndike, and Dill (1935) demonstrated that metabolic rate was elevated 25% 15 hours after playing football for 2 hours. The production of an extended elevation of metabolic rate may require exercise of moderate to severe intensity. Pacy, Barton, Webster, and Garrow (1985) found that subjects exercised at 35-55% of maximum capacity for 20 minutes each hour for four hours had a normal rate of oxygen uptake one hour after exercise. Metabolic rate is also increased in rats that exercise by swimming compared to controls (Hill, Davis, & Tagliaferro, 1983). The second way exercise increases caloric expenditure occurs during dieting. Restricted caloric intake reduces resting metabolic rate (RMR) (Bray, 1969; Donahoe, Lin, Kirschenbaum, & Keesey, 1984; Nieman et al., 1988). Exercise counters this diet-induced drop in metabolic rate (Donahoe et al., 1984; Nieman et al., 1988). The third way that exercise may contribute to energy expenditure is by increasing the thermic effect of food (TEF). The thermic effect of food is the increase in metabolism that occurs immediately following food consumption. Swimming increased resting metabolic rate independently of dietary conditions, but increased TEF in the rats eating a cafeteria diet, but did not increase TEF of rats eating laboratory chow (Hill et al., 1983).

Therefore, exercise may attenuate or prevent weight gain during nicotine cessation by increasing metabolic rate

for extended periods of time following the exercise session. Exercise may also prevent weight gain by increasing the thermic effect of food, and in individuals who restrict their caloric intake to avoid the weight gain, exercise should prevent the drop in RMR that usually accompanies caloric restriction. Although the actual caloric cost of an exercise session may not be large enough to significantly affect body weight, by adding the increased energy expenditure following a meal and the increased energy expenditure for extended periods of time after exercise, the total energy expenditure increase may be large enough to significantly reduce body weight or prevent weight gain during cessation of nicotine.

Insulin, Nicotine, and Exercise

Insulin may mediate nicotine's effects on body weight. Two weeks of nicotine administration to rats via osmotic minipumps significantly lowers plasma insulin levels (Grunberg, Popp et al., 1988). In contrast, during nicotine cessation, plasma insulin levels increase somewhat over control levels (Grunberg, Raygada et al., 1988). Insulin administration results in increased food consumption and weight gain in rats (Booth, 1972; Brandes, 1977; Panksepp et al., 1975), and increased consumption of carbohydrates by rats (Kanarek, Marks-Kaufman, & Lipeles, 1980). Rodin, Wack, Ferrannini, and DeFronzo (1985) reported that human subjects with high plasma insulin levels (independent of plasma glucose levels) increased hunger ratings, palatability ratings of sweet solutions, and liquid food intake. Based on the

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findings regarding nicotine and insulin and the studies of insulin and food intake, it has been postulated that changes in insulin underlie the effects of nicotine and cessation of nicotine on body weight and specific food consumption (Grunberg, Popp et al., 1988).

Exercise has both acute and chronic effects on plasma insulin levels that are opposite to the effects of nicotine cessation on insulin and, therefore, may be particularly useful in preventing or attenuating the body weight and specific food consumption changes that occur when smokers quit smoking. Exercise reduces plasma insulin levels in both obese (Bjorntorp, de Jounge, Sjostrom, & Sullivan, 1970) and non-obese (LeBlanc et al., 1972) humans. Also, well-trained men have lower plasma insulin levels than sedentary men (Bjorntorp et al., 1972). Acute effects of exercise also include a reduction of plasma insulin levels (Galbo, Holst, & Christensen, 1975; Galbo, Richter, Holst, & Christensen, 1977). Because exercise reduces insulin levels both acutely and chronically, exercising may prevent or attenuate changes in eating behavior and body weight that occur during nicotine cessation.

Summary

Based on the available literature, cigarette smoking causes serious health problems in a large number of people. In spite of these well-known health effects of cigarette smoking, close to 29% of the adult population in the United States smokes. One reported reason smokers do not quit

smoking is the fear of the weight gain associated with quitting smoking. Therefore, determining ways to minimize or prevent this weight gain may increase the likelihood that smokers will quit smoking and thereby reduce the health consequences currently attributed to cigarette smoking.

A large component of weight gain during nicotine cessation can be attributed to increases in sweet food consumption. However, because body weight changes still occur during nicotine administration when only bland food is available and bland food consumption is not affected by nicotine administration and cessation, changes in energy expenditure also must contribute to the inverse relationship between nicotine and body weight. Restricting access to sweet food attenuates weight increases during nicotine cessation. However, human smokers generally have access to sweet foods, and this restriction may be difficult for smokers trying to quit smoking. A behavioral or pharmacological manipulation that reduces appetite for sweet foods should help smokers who stop smoking reduce their sweet food consumption and prevent weight gain during nicotine cessation.

Exercise may prevent weight gain during nicotine cessation through its effects on both food intake and energy expenditure. Exercise reduces plasma insulin levels. Because high insulin levels are associated with increased hunger and pleasantness of sweet tastes, by preventing or minimizing the increase in plasma insulin levels found during nicotine cessation, exercise may prevent the increase in sweet food

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consumption during nicotine cessation. Exercise increases energy expenditure and also may counter changes in energy expenditure associated with nicotine cessation.

The present study was designed to determine the effects of exercise and the availability of sweet food on food consumption and body weight during nicotine cessation. Plasma insulin levels also were measured to determine if these levels were consistent with predicted levels based on the hypothesis that insulin is a biochemical mechanism for changes in body weight during nicotine cessation.

Overview

This study used an animal model to examine whether exercise and restricted access to sweet food prevent the excessive weight gain that usually follows nicotine cessation. This study also examined the effects of exercise on sweet and bland food consumption during nicotine cessation. There were three phases to the experiment: baseline, drug administration (nicotine or saline), and drug cessation. The experiment used a 2 (nicotine vs. saline) x 2 (sweet and bland food vs. bland food) x 3 (sedentary vs. low exercise vs. high exercise) factorial design. Half of the rats had access to both bland laboratory chow and sucrose-sweetened laboratory chow throughout the study, and the other rats had access to only the bland laboratory chow.

Following one week of collecting baseline measurements of body weight, food, and water consumption, either nicotine or a saline solution was administered continuously for two

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weeks via an osmotic minipump. The food, water, and body weight measurements were continued during both the two-week drug administration period and the five-week period of nicotine cessation.

Following the removal of the osmotic minipumps, two thirds of the rats in each of the four drug x food conditions swam six days per week for five weeks. The low exercise group swam for half of the time that the high exercise group swam each day. After the exercise and drug cessation phase, the rats were sacrificed and blood samples, muscle samples, and the right epididymal and retroperitoneal and peri-renal fat pads were collected.

Insulin was hypothesized to be the mechanism underlying the increased consumption of sweet food, and consequently the weight gain, during nicotine cessation. Because exercise lowers insulin levels and could prevent the rise in insulin during nicotine cessation, preventing the resulting weight gain, circulating plasma insulin levels were measured in addition to body weight and food consumption. A citrate synthase assay was performed on the muscle samples to examine physiological training effects. The fat pads were weighed and expressed as a percentage of total body weight to provide an index of body fatness. This index is important in distinguishing possible weight gain associated with increasing muscle mass in response to exercise from weight gain associated with increasing body fat during nicotine cessation.

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Hypotheses

Baseline Period: Body Weight and Food Intake.

1) Rats with access to sweet food will weigh more than those having access to only bland food.

2) Rats with access to sweet food will consume less bland food than rats having only bland food.

3) Total caloric intake of rats with access to sweet food will be greater than that of rats with only bland food.

During Drug Administration: Body Weight.

1) Rats that have access to sweet food and receive saline will have the highest body weights.

2) Rats that have only access to bland food and receive nicotine will have the lowest body weights.

3) Rats that have access to sweet food and receive nicotine and rats that have access to only bland food and receive saline will have intermediate values for body weight.

During Drug Administration: Food Intake.

1) All rats with access to only bland food will have similar bland food consumption values.

2) Animals with access to sweet food that also receive nicotine will have bland food consumption values that are similar to those for all rats having access to only bland food.

3) Rats that have access to sweet food and receive saline will have the lowest bland food consumption values.

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4) Rats receiving nicotine will consume less sweet food than rats receiving saline.

5) Rats with access to sweet food that receive saline will consume the greatest number of calories.

Drug Cessation Period: Body Weight.

1) Sedentary rats with access to sweet food and that received saline during the drug administration period will have the highest body weights.

2) Exercising rats with access to only bland food and that received nicotine during the drug administration period will have the lowest body weights.

3) Exercise will reduce body weight gains compared to the sedentary rats within each of the drug and diet conditions.

4) Rats with access to sweet food will have higher body weights than rats with access to only bland food within each of the four drug and activity conditions.

5) Rats that had received nicotine during the drug administration period will have lower body weights after cessation than rats that had received saline within each diet and activity group. This effect will be reduced in sedentary rats that had received nicotine with access to sweet food because of their larger rate of weight gain during cessation.

Drug Cessation Period: Food Intake.

1) All rats with access to only bland food will have similar food consumption values. However, exercising rats may have slightly lower food consumption values.

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2) All rats with access to sweet food will consume similar amounts of bland food. However, exercising rats may have slightly lower food consumption values.

3) Sedentary rats that had received nicotine will have the highest sweet food consumption.

4) Exercise will reduce sweet food consumption compared to values for sedentary rats for each drug group. This effect will be greatest in the rats that received nicotine during the drug administration period.

5) Rats that received nicotine during the drug administration period will consume more sweet food than rats that received saline within each exercise group.

6) Sedentary rats that received nicotine will have the highest caloric consumption.

7) Exercise will reduce total caloric consumption compared to sedentary values for each drug group.

8) Rats that received nicotine during the drug administration period will have higher caloric intakes than rats that received saline within each exercise group.

9) Rats with access to sweet food will have higher caloric intakes than rats that only had access to bland food within each drug and exercise group.

Drug Cessation: Physiological Measures.

1) Rats with access to sweet food will have higher plasma insulin levels than rats with access to bland food within each drug and exercise group.

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2) Rats that received nicotine during the drug administration period will have higher plasma insulin levels than rats receiving saline within each diet and exercise group.

3) Exercising rats will have lower plasma insulin levels than sedentary rats within each drug and diet group.

4) Exercising rats will have higher muscle tissue levels of citrate synthase than sedentary rats.

5) Rats with access to sweet food will have fat pads contributing a higher percentage of their total body weight than rats with access to bland food within each drug and exercise group.

6) Rats that received nicotine will have fat pads contributing a higher percentage of their total body weight than rats that received saline within each diet and exercise group.

7) Exercising rats will have fat pads contributing a lower percentage of their total body weight than sedentary rats within each drug and diet group.

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METHODS

Subjects

The subjects were 96 male Sprague-Dawley rats weighing approximately 300 g (3 - 4 months old) purchased from Hilltop Laboratory Animals (Scottdale, PA). Previous studies have found that changes in body weight and food consumption during and after nicotine administration in rats parallel results of human smokers and smokers who quit smoking (Grunberg, 1982; 1988). An animal model for smoking cessation provided greater control over diet and physical activity manipulations than human smokers would have provided. The animals were individually housed in standard polypropylene shoebox cages (35.6 x 15.2 x 20.3 cm) fitted with metal grill lids. Elevated floors were placed on the floors of the cages above absorbent wood Pine-Dri shavings to reduce both copraphagia and the amount of wood shavings in the food cups. The animals were housed in a room with overhead flourescent illumination providing a twelve hour light/dark cycle, and the room was maintained at approximately 25 degrees C and 50% relative humidity. Tap water and either one or two kinds of rat chow (see below for detailed descriptions) were continuously available except during daily measurements or exercise periods.

Drug Administration

Rats were anesthetized with methoxyflurane and Alzet miniosmotic pumps (Model 2002) were implanted SC between the shoulders to deliver nicotine or saline at a constant rate of

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0.5 ul/h for 14 days. Methoxyflurane is a short-acting inhalational anesthetic and is, therefore, appropriate for the brief and minor procedure of pump implantation. Physiological saline was used to make the nicotine solutions (with nicotine dihydrochloride) and served as the control solution. Equal numbers of rats received saline or 12 mg/kg body weight/day. Drug dosage was computed as nicotine base. This dosage of nicotine was used in previous research and reliably induced changes in body weight of rats that parallel changes in body weight of human smokers who quit smoking (Grunberg, 1982; Grunberg et al., 1984; Grunberg et al., 1985). Miniosmotic pumps were used to provide animals with drug each day without the trauma of daily injections, and to establish and maintain fairly constant plasma levels of nicotine for many days.

Foods

Food was available in two stainless steel food cups in each cage. Half of the rats received Charles River RMH 3200 meal (a standard laboratory chow) in both food cups. The other half of the rats received one cup filled with the meal and the other cup filled with a sweet food composed of 40% sucrose and 60% meal (by weight). This combination of sucrose and meal resulted in consistent weight gain in rats throughout a prior study, and lower consumption rates during nicotine administration compared to consumption before and after drug administration (Sibolboro & Grunberg, 1989). The bland laboratory chow provided 3.50 kilocalories per gram and the sweetened chow provided 3.77 kilocalories per gram. These

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foods were available at all times except during exercise and measurement sessions. In addition, food was removed from the cages 4 hours prior to both pump implantation and removal, and returned to the cages approximately 4-6 hours after the surgical procedures to avoid conditioned taste aversion to either type of food (Garcia, 1955). Food was also removed from the cages 12 hours prior to decapitation and the collection of the blood sample to obtain fasting levels of plasma insulin. Fasting insulin levels were chosen to avoid the variablility induced by consumption of differing amounts of food and sugar content just prior to blood collection.

Exercise

Rats were exercised by swimming in groups of six in a large (32 gal.) plastic trash can (diameter = 26 cm, water depth = 40-45 cm) for 6 days/week. The high exercise group swam for periods of time up to and including 60 minutes, and the low exercise group swam for periods up to and including 30 minutes. This paradigm was based on the work of Savard, Palmer, and Greenwood (1986) and Taguchi, Hata, and Itoh (1985) in which swimming was an effective exercise manipulation for rats. The swimming procedure was chosen over other forms of exercise, such as treadmill running, because pilot studies indicated that a higher percentage of rats would swim than would run on a treadmill. Because of this difference between the two exercise procedures, swimming should have resulted in less variance in the exercise manipulation, as well as less variance in the stress of the

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procedure among animals due to differential exposure to the shock grid used in standard treadmill exercise procedures.

The water was 37 degrees C at the start of each swimming period and was allowed to cool until the end of the swimming period for each set of six rats. Isothermic water was used to start each swimming session in order to minimize the possible thermogenic effects of cold water that could affect energy expenditure. This temperature was used because it has been reported to allow for longer swimming times for rats (Baker & Horvath, 1964). (See Appendix 1 for specific details concerning the training schedule.)

Rats that were not in the swimming conditions were dipped in the water, wiped with towels to dry them, and placed back in their home cages. The dipping procedure was used to control for the thermogenic effects of the exposure to water and the effects of its evaporation on body heat loss.

Physical Activity Monitoring

Physical activity was monitored during the cessation phase of the study to determine if the exercise procedure reduced general activity levels outside of the exercise sessions, which could reduce the effectiveness of the exercise procedure in preventing weight gain during nicotine cessation. Physical activity was also monitored during the baseline and drug administration phases to establish a baseline for each monitored animal, and to determine if diet or nicotine administration affected physical activity. The two median weight rats from each of the twelve groups were selected for

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the monitoring procedure. Each animal was placed in a 40.6 cm square Plexiglas cage inside a Digiscan Optical Digital Sensor Activity Monitor that was equipped with a Datalogger 8000 data collection device (Omnitech Electronics Inc., Columbus, OH) approximately two hours prior to the beginning of the dark cycle. Each of these rats was monitored throughout the 12-hour dark cycle one day during the baseline phase, one day during the first week of drug administration, one day during the second week of the cessation phase, and one day during the final week of the cessation phase. The monitors recorded one unit of horizontal activity each time an animal crossed a beam of infrared light within an 8 x 8 grid of beams 2.5 cm above the floor. Vertical activity was recorded by a second set of beams 15 cm above the floor. The Dataloggers printed an hourly total of horizontal activity units and vertical activity units for each animal during each of the twelve hours monitored in a session. The mean number of horizontal units of activity per hour was selected as the measurement for physical activity in each phase of the study, because most rats displayed very little vertical activity.

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Daily measurements

Body weight, food, and water consumption were measured daily. Body weight measurements were made with a Sartorius programmable electronic balance and a Sartorius printer/programmer. The balance was programmed to calculate body weight as a mean based on multiple weighings made at one second intervals to minimize effects of the physical activity

of the rats on the measurements. Body weight was measured to the nearest tenth of a gram and food and water consumption were measured to the nearest one hundredth of a gram. Food cups and water bottles were filled when necessary to ensure that an excess of food and water was available at all times, and were changed once each week. Food cups were placed at one end of each cage and were switched daily to avoid any position preference effects.

Insulin

Approximately 36 hours after the last exercise period, the rats were decapitated and trunk blood was collected through a funnel rinsed with heparinized saline (500 IU/ml) into plastic test tubes (14 ml) with 100 ul of a heparin solution (10,000 IU/ml) in them to prevent coagulation. Samples were put on ice immediately after collection and then were centrifuged at 3000 rpm for 20 minutes. The plasma was transferred to clean test tubes and placed in a freezer and stored at -70 degrees C until the insulin assay was performed. Insulin levels were determined with a competitive-binding radioimmunoassay procedure from a prepared RIA kit (Radioassays Systems Laboratories, Inc.).

Citrate Synthase

Immediately after decapitation, each right gastrocnemius muscle was completely removed and immediately frozen in liquid nitrogen. These samples were stored at -70 degrees C until assayed for citrate synthase activity. A horizontal section (approximately 40 mg) of the muscle tissue

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was minced and homogenized in cold 175 mM KCl at pH 7.4. Homogenates were frozen and thawed three times to disrupt the mitochondrial membranes. Citrate synthase was then assayed by the spectrophotometric method of Srere (1969) using 5,5-dithiobis-(2-nitrobenzoate). (See Appendix 2 for detailed assay procedure.)

Procedure

After an initial gentling period, rats were divided into twelve groups of eight rats with each group having a similar mean, median, and range for body weight. Body weight and consumption of each of the foods and water was measured daily. The baseline period for data collection lasted six days. On the seventh day, all rats had Alzet miniosmotic pumps filled with either a saline or a nicotine solution implanted. The pumps remained inside the rats and daily measurements were continued for fourteen days. On the fifteenth day of drug administration, the pumps were removed. Triple antibiotic ointment was smeared on the incisions after both the first and second surgeries to reduce the risk of infection. During the first few days of the swimming phase all rats had the ointment applied daily. The rats were allowed to rest on the day following pump removal. The exercise procedure began on the second day after pump removal and was continued, along with the daily measurements, for twenty-seven days. Thirty-six hours after the last exercise period, the rats were decapitated and blood, muscle, and fat samples were collected.

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RESULTS

Data Analysis Overview

The data hypothesized to be related to weight changes were analyzed three ways in order to obtain information about the data that was not available with any single analysis. First, daily measures were analyzed with repeated measures ANOVAs for each daily measurement. The repeated measures ANOVAs provided information concerning changes in these measurements across the three phases of the study. Next, three MANOVAs were performed to assess the effects of diet, drug, and exercise on cessation phase measurements, including plasma insulin levels and the fat index, because these two variables were only available at the end of the cessation phase. In addition, multiple regression analyses were performed to determine the relative contribution of plasma insulin levels, as well as the diet, drug, and exercise manipulations, to changes in body weight and food consumption variables. Each type of analysis is described in more detail below and is then followed by the presentation of the results of these analyses. All post hoc analyses were based on two-tailed tests of significance, and the alpha level was set at .05. Only statistically significant findings concerning the major hypotheses of this study are reported in the text. (See Appendix 3 for detailed statistics for the repeated measures ANOVAs and MANOVAs.)

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Repeated Measures Analyses

All daily measures (body weight, sweet food consumption, bland food consumption, and water consumption) were analyzed with separate repeated measures ANOVAs. A multivariate criterion (Pillai's trace) was used to determine if diet, drug, and exercise manipulations influenced the overall pattern of changes in the daily measurements during the study. Pillai's trace criterion was used because of its robustness for violations of homogeneity of variance (Harris, 1985). Five-day means during the baseline phase, the second week of the drug administration phase, and the final week of the drug cessation phase were used for each of these variables in the analyses. Significant multivariate tests for each dependent variable across phases of the study were followed by ANOVAs for contrasts between the cessation period and the other two periods of the study. These two contrasts were selected because this study was primarily concerned with effects of the independent variables during the cessation period. However, ANOVAs for contrasts between baseline and drug administration were also performed to assess main effects and interactions resulting from nicotine administration.

Two additional multivariate repeated measures ANOVAs were performed to determine the effects of the three independent variables on 5-day means during the same periods used for the daily measurement variables for total daily caloric consumption, and for food efficiency measurements during the drug administration and cessation periods. The

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food efficiency variables were used to examine the effects of the independent variables on the tendency to gain weight (a balance of energy absorption and energy expenditure), controlling for starting body weight and the number of kilocalories consumed. The first food efficiency variable was defined as the change in body weight from baseline to the second week of the drug administration period per kilocalorie of food consumed between the baseline and drug administration measurements, per gram of baseline body weight. The second food efficiency variable was computed in a similar manner for the period between drug administration and drug cessation. Increased food efficiency indicated an increased tendency to gain weight for a given caloric intake. Post hoc tests (Scheffe's) were used for each dependent variable when appropriate.

Body weight. Figures 1-3 present the body weight data during the three phases of the study. Figure one presents body weight data for all twelve groups, while Figures 2 and 3 present the same data with groups collapsed across exercise and diet conditions, respectively. Drug and exercise manipulations, as well as a diet and drug interaction, influenced the overall pattern of body weight changes during the three phases of the study [$F(2,76) = 104.49$, $p<.0001$; $F(4,154) = 11.49$, $p<.0001$; and $F(2,76) = 3.73$, $p<.03$; respectively]. Nicotine administration reduced the rate of weight gain during the drug administration period compared to saline controls [$F(1,77) = 157.80$, $p<.0001$]. Although rats

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that had received nicotine gained weight at a faster rate during the cessation period than saline controls [$F(1,77) = 9.64$, $p<.003$], their overall rate of weight gain between baseline and the last week of cessation remained lower than in the saline controls [$F(1,77) = 12.89$, $p<.0006$].

Exercise reduced the rate of weight gain between drug administration and the last week of cessation [$F(2,77) = 26.56$, $p<.0001$]. Exercise also reduced the overall rate of weight gain between baseline and drug cessation phases [$F(2,77) = 14.03$, $p<.0001$], as was hypothesized. Post hoc Scheffe tests indicated that the high exercise group weighed less than the sedentary group at the .05 level. Although the low exercise group had body weights intermediate between the sedentary and high exercise groups, the body weights of the low exercise group were not statistically different.

The diet x drug interaction between baseline and drug administration phases suggested that rats having access to both sweet food and bland food tended to gain less weight between baseline and the drug administration phase than rats having access to only bland food if these rats received nicotine, but not if they received saline. This interaction approached significance based on the univariate ANOVA [$F(1,77) = 3.58$, $p<.07$]. No such interaction existed during the cessation period, possibly because of the influence of exercise. Having access to sweet food resulted in lower rates of weight gain from baseline to cessation in exercising rats that had received nicotine, but not in exercising rats that

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received saline. Sedentary rats that had received nicotine gained more weight if they had access to both sweet and bland food than if they only had access to bland food. This relationship was not present in rats that had received saline. This tendency toward a three-way interaction (diet x drug x exercise) for contrasts between the baseline and cessation period body weights approached statistical significance [$F(2,77) = 3.01$, $p<.06$].

In summary, nicotine administration reduced rate of weight gain and nicotine cessation increased rate of weight gain as hypothesized. In contrast to hypothesized results, access to sweet food did not increase rate of saline or nicotine-induced weight gain. There was a tendency for access to sweet food to result in lower rates of weight gain during nicotine administration, which was consistent with the findings of Grunberg, (1982) and Grunberg, Bowen et al. (1985). Access to sweet food also slightly reduced rate of weight gain in exercising rats that had previously received nicotine. In addition, exercise reduced rate of weight gain.

Because diet interacted with nicotine administration and exercise in an unexpected manner, some of the hypotheses concerning the rank order of groups according to body weight were not supported. During the drug administration phase, groups receiving saline were similar in body weight, regardless of diet condition. Rats receiving nicotine with access to only bland food weighed less than rats receiving saline, but weighed more than rats with access to sweet food

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receiving nicotine. During the cessation phase, sedentary rats that had received saline and only had access to bland food weighed more than any other group, while exercising rats that had received nicotine and had access to sweet food weighed less than any other group.

Daily caloric consumption. Figures 4-6 present daily caloric consumption during the three phases of the study. Figure 4 presents caloric consumption for all 12 groups of rats accross the three phases of the study. Figures 5 and 6 present the same data collapsed across exercise and diet conditions, respectively. Pillai's trace criterion indicated that the diet, drug, and exercise manipulations influenced the overall pattern of distribution of daily caloric consumption during the three phases of the study [$F(2,76) = 17.47$, $p < .0001$; $F(2,76) = 20.82$, $p < .0001$; and $F(4,154) = 2.54$, $p < .05$; respectively].

Although the sweet food provided more calories per gram than the bland food, and access to sweet food increased daily caloric consumption during the baseline phase [$F(1,77) = 10.47$, $p < .002$], having access to sweet food reduced daily caloric consumption during the drug cessation period relative to the baseline period [$F(1,77) = 19.33$, $p < .0001$]. During the period between drug administration and cessation, rats that had received saline and had access to sweet food reduced their caloric consumption, while the other three diet x drug groups increased their caloric consumption. This interaction was statistically significant [$F(1,77) = 4.51$, $p < .04$].

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Nicotine administration reduced daily caloric consumption [$F(1,77) = 40.57$, $p<.0001$], and nicotine cessation increased caloric consumption compared to the drug administration phase [$F(1.77) = 11.17$, $p<.002$]. Exercise also reduced caloric consumption from baseline to cessation, and from drug administration to cessation, compared to sedentary groups [$F(2,77) = 4.88$, $p<.02$; and $F(2,77) = 4.56$, $p<.02$, respectively].

In summary, access to sweet food increased caloric intake during the baseline period, as was hypothesized. However, access to sweet food reduced caloric intake during the drug cessation period relative to baseline and, also in contrast to hypothesized results, rats that had access to sweet food and were receiving saline did not consume the largest number of calories during the drug administration phase. In addition, sedentary rats that had received nicotine did not consume more calories than all other rats as hypothesized, possibly because their body weights remained lower than saline controls. Exercise reduced caloric consumption as hypothesized.

Bland food consumption. Figures 7-9 present bland food consumption data during the three phases of the study. Figure 7 presents bland food consumption of all 12 groups across the three phases of the study. Figures 8 and 9 present the same data collapsed across exercise and diet conditions, respectively. Pillai's trace criterion indicated that diet, exercise, and a diet x drug interaction influenced the overall

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pattern of bland food consumption during the three phases of the study [$F(2,76) = 18.81$, $p<.0001$; $F(4,154) = 2.45$, $p<.05$; and $F(2,76) = 5.38$, $p<.007$; respectively].

Access to sweet food markedly reduced bland food consumption across all phases [$F(1,77) = 1576.60$, $p<.0001$; $F(1,77) = 436.08$, $p<.0001$; and $F(1,77) = 1204.31$, $p<.0001$; respectively]. Contrasts between the baseline and drug administration periods, and between drug administration and cessation periods indicated that a diet x drug interaction affected bland food consumption [$F(1,77) = 9.85$, $p<.003$ and $F(1,77) = 6.41$ $p<.02$ respectively]. Rats that had access to both sweet and bland food tended to increase bland food consumption from baseline to drug administration periods, while rats with access to only bland food tended to decrease or maintain their levels of bland food consumption from baseline to drug administration. These effects of access to sweet food on bland food consumption were stronger for the rats receiving nicotine than for those receiving saline.

Exercise tended to prevent or reduce the increase in bland food consumption from baseline to cessation. However this effect failed to reach significance [$F(2,77) = 2.93$, $p< .06$]. Contrasts between drug administration and drug cessation phases also indicated that exercise reduced bland food consumption $F(2,77) = 4.05$, $p<.03$]. Post hoc Scheffe tests indicated that the low exercise rats consumed less bland food than the sedentary rats during the cessation phase.

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In summary, access to sweet food reduced bland food consumption across all three phases of the study. Access to sweet food resulted in increasing levels of bland food consumption, while access to only bland food decreased or maintained levels of bland food consumption from baseline to drug administration phases. This effect was strongest for the rats receiving nicotine, resulting in an interaction between diet and drug manipulations. Exercise reduced bland food consumption, and may reduce caloric consumption and cessation weight gain by this effect on bland food consumption.

As hypothesized, rats that only had access to bland food consumed similar amounts of bland food, regardless of drug and exercise conditions. However, exercise slighly reduced bland food consumption relative to sedentary rats with access to only bland food. Although nicotine administration slightly increased bland food consumption in rats with access to sweet food as hypothesized, bland food consumption for these rats did not approach the bland food consumption for rats with access to bland food alone. Rats receiving saline that had access to sweet food had the lowest bland food consumption during the drug administration phase. During the cessation phase, rats with access to bland food alone had similar bland food consumption levels and exercise slightly lowered these levels. Rats with access to sweet food had similar levels of bland food consumption as hypothesized, but exercise did not affect bland food consumption in these rats, possibly because their bland food consumption was very low.

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Sweet food consumption. Figures 10 and 11 present sweet food consumption data during the three phases of the study. Figure 10 presents sweet food consumption for all 6 groups, while Figure 11 presents the same data collapsed the exercise condition. According to Pillai's trace criterion, the drug manipulation influenced overall patterns of sweet food consumption during the three phases of the study [$F(2,39) = 9.54$, $p<.0005$]. Nicotine administration resulted in greater reductions in sweet food consumption than in saline controls from baseline to drug administration periods [$F(1,40) = 13.98$, $p<.0007$], and somewhat attenuated the reduction in sweet food consumption between baseline and cessation periods [$F(1,40) = 3.31$, $p<.08$]. Nicotine cessation increased sweet food consumption from drug administration phase values [$F(1,40) = 16.09$, $p<.0004$]. Also, exercise tended to reduce sweet food consumption to a greater extent than in sedentary controls between baseline and cessation periods [$F(2,40) = 2.49$, $p< .10$].

In summary, nicotine administration greatly reduced sweet food consumption, and nicotine cessation increased sweet food consumption compared to saline controls, as hypothesized. Exercise slightly reduced sweet food consumption. Also, sedentary rats that had received nicotine consumed the most sweet food, as hypothesized.

Water consumption. Figures 12 and 13 present water consumption data for the three phases of this study. Figure 12 presents water consumption for all 12 groups, while Figure

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13 presents the same data collapsed across exercise conditions. Pillai's trace criterion indicated that diet and drug manipulations influenced overall patterns of water consumption during the three phases of the study [$F(2,76) = 4.37$, $p<.02$; and $F(2,76) = 12.98$, $p<.0001$; respectively]. Although the diet manipulation did not affect changes in water consumption across phases of the study, access to sweet food reduced water consumption during baseline, drug, and cessation phases [$F(1,77) = 43.20$, $p<.0001$; $F(1,77) = 40.75$, $p<.0001$; and $F(1,77) = 50.18$, $p<.0001$; respectively]. Nicotine administration reduced water consumption between the baseline and drug administration phases of the study compared to saline controls ($F(1,77) = 19.20$, $p<.0001$), but not between baseline and cessation.

In summary, access to sweet food and nicotine administration reduced water consumption.

Food efficiency. Figures 14-16 present food efficiency values (tendency to gain weight controlling for initial body weight and kilocalories consumed) computed between baseline and drug administration and between drug administration and cessation phases. Figure 14 presents food efficiency for all 12 groups, while Figures 15 and 16 present the same data collapsed across exercise and diet conditions, respectively. According to Pillai's trace criterion, drug and exercise manipulations and a diet x drug interaction influenced the overall pattern of food efficiency [$F(1,77) = 122.94$, $p<.0001$; $F(2,77), = 5.51$, $p<.006$; and $F(1,77) = 5.51$,

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p<.03; respectively]. Because baseline food efficiency values were not available, separate ANOVAs for the drug administration and cessation food efficiency variables (rather than contrasts between the two) were performed. During drug administration, nicotine reduced food efficiency [$F(1,77) = 88.50$, $p<.0001$]. and during cessation, prior nicotine administration increased food efficiency [$F(1,77) = 24.73$, $p<.0001$] compared to saline controls. Exercise reduced food efficiency [$F(2,77) = 29.13$, $p<.0001$]. During the drug administration phase, access to sweet foods decreased food efficiency in animals receiving nicotine, but not in those receiving saline [$F(1,77) = 5.38$, $p<.03$].

In summary, nicotine administration and exercise reduced food efficiency. In other words, both nicotine administration and exercise reduce weight gain independently of their effects on food intake. In contrast, nicotine cessation increased food efficiency, or tendency to gain weight per kilocalorie consumed. Access to sweet foods decreased food efficiency in rats receiving nicotine. This last finding is surprising in light of the hypotheses concerning the effects of access to sweet food on plasma insulin levels and weight gain. If access to sweet food increased plasma insulin levels during nicotine administration relative to the rats with access to only bland food, body weight would be higher than in rats with access to only bland food, instead of lower than in rats with restricted access to sweet food.

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MANOVAs - cessation phase

The second method of data analysis used three-factor MANOVAs (2 x 2 x 3) to determine if diet, drug cessation, and exercise affected various combinations of the dependent variables during the drug cessation phase, including plasma insulin levels and an index of fatness, which were only available for the drug cessation period. The fat index was computed by dividing the total weight of the fat pads that were removed after decapitation by cessation phase body weight and multiplying by 100 to express the value as a percentage. Three separate MANOVAs were used to analyze the data. The dependent variables for the first MANOVA included: weight change between drug administration and drug cessation periods, plasma insulin levels, fat index, daily caloric consumption, and water consumption. The second MANOVA included all of the dependent variables of the first MANOVA except total caloric consumption. Sweet food consumption and bland food consumption replaced total caloric consumption in this analysis and permitted the assessment of the influence of the independent variables on relative consumption of the two types of food in rats that had access to both foods. The third MANOVA included plasma insulin levels, fat index, and the food efficiency measure for the time period between drug administration and drug cessation. Food efficiency was not used in the other two MANOVAs because it was calculated from several of the dependent variables in those analyses. Again, Pillai's trace criterion was used to assess multivariate

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differences. Univariate F tests and Sheffe's post-hoc tests were performed for each dependent variable when appropriate.

The first MANOVA included the following dependent variables: change in body weight from drug administration to drug cessation, plasma insulin levels, fat index, daily caloric intake, and water consumption during the cessation phase. Pillai's trace criterion indicated that diet, drug, and exercise affected the overall pattern of these dependent variables [$F(5,73)= 12.07$, $p<.0001$; $F(5,73) = 8.75$, $p<.0001$; and $F(10,148) = 5.44$, $p<.0001$; respectively]. Therefore, ANOVAs were performed for each dependent variable to determine which of these variables were affected by the diet, drug, and exercise manipulations.

Nicotine administration increased the amount of weight gained between the drug administration and cessation phases [$F(1,77) = 9.64$, $p<.003$], while exercise reduced the amount of weight gain during this period [$F(2,77) = 26.56$, $p<.0001$]. Post hoc Scheffe's indicated that all exercise groups (sedentary, low, and high) differed from each other in cessation body weight gain. (See Figure 17.)

Access to sweet food increased plasma insulin levels [$F(1,77) = 5.18$, $p<.03$], while exercise reduced plasma insulin levels [$F(2,77) = 4.13$, $p<.02$]. Post hoc Scheffe's indicated that only the plasma insulin levels for rats in the sedentary and high exercise groups differed. (See Figure 18.)

Access to sweet food increased fat indices [$F(1,77) = 5.91$, $p<.02$], while prior nicotine administration reduced

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fat indices [$F(1,77) = 5.17$, $p<.03$]. Exercise also reduced fat indices [$F(2,77) = 7.10$, $p<.002$]. Scheffe's post hoc tests indicated that only the sedentary and high exercise groups differed. (See Figure 19.)

Access to sweet food and exercise reduced daily caloric consumption during cessation [$F(1,77) = 8.98$, $p<.004$; and $F(2,77) = 3.29$, $p<.05$; respectively]. Post hoc Scheffe's tests indicated that only the sedentary and low exercise groups differed from each other. As was reported in the repeated measures analyses, access to sweet food reduced water consumption during cessation [$F(1,77) = 50.18$, $p<.0001$].

In summary, prior nicotine administration increased the amount of weight gain between the end of the drug administration and cessation phases. In spite of the greater gain in weight, rats that had received nicotine remained leaner than controls. Exercise reduced cessation weight gain, as well as proportion of body fat. Exercise also reduced plasma insulin levels and daily caloric consumption. Although diet did not affect cessation weight gain, access to sweet foods increased proportion of body fat and plasma insulin levels. In contrast, access to sweet food reduced daily caloric intake and water consumption during the cessation phase compared to rats with access to only bland food.

The second MANOVA included the following dependent variables: body weight change between drug administration and cessation, insulin levels, fat index, sweet food consumption, bland food consumption, and water consumption. This MANOVA

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was used to assess effects of the independent variables on specific food consumption, rather than general caloric consumption. Again, the drug and exercise manipulations affected the overall pattern of distribution of the dependent variables according to Pillai's trace criterion [$F(6,35) = 4.91$, $p<.001$; and $F(12,72) = 3.18$, $p<.002$; respectively]. Diet was not an influence, because only those rats with access to sweet food were used in this analysis. Therefore, ANOVAs were performed for each dependent variable to determine which of these variables was affected by the drug and exercise manipulations.

As in the previous MANOVA, nicotine administration increased cessation weight gain [$F(1,40) = 8.43$, $p<.007$], while exercise reduced cessation weight gain [$F(2,40) = 14.81$, $p<.0001$]. Post hoc Scheffe's tests indicated that both exercise groups differed from the sedentary rats, but not from each other in cessation weight gain.

Exercise reduced plasma insulin levels [$F(2,40) = 4.58$, $p<.02$] and proportion of body fat [$F(2,40) = 5.65$, $p<.007$]. Post hoc Scheffe tests indicated that only the sedentary and high exercise groups differed from each other in insulin levels and in proportion of body fat. Prior nicotine administration did not reduce proportion of body fat in rats that had access to sweet food. Also, drug and exercise manipulations did not affect sweet or bland food consumption, or water consumption during the cessation phase in rats with access to sweet food. Therefore, exercise

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reduced bland food consumption primarily in rats with access to only bland food.

In summary, prior nicotine administration increased cessation weight gain in rats with access to sweet food. Exercise reduced cessation weight gain, plasma insulin levels, and proportion of body fat. Also, access to sweet food resulted in proportions of body fat that were similar for rats that received nicotine and saline controls, indicating that nicotine's reduction of fat indices only occurred in rats with access to only bland food. Therefore, restricting access to sweet food prevents proportion of body fat in nicotine-treated rats from reaching the proportions of fat for saline controls during nicotine cessation.

The third MANOVA included the following dependent variables: food efficiency between drug administration and cessation, plasma insulin levels, and fat index. Water consumption was not included in this MANOVA because only diet affected water consumption during cessation, and diet did not affect cessation weight change. Pillai's trace criterion indicated that diet, drug, and exercise affected the overall pattern of distribution of these dependent variables [$F(3,75) = 3.11$, $p<.04$; $F(3,75) = 12.81$, $p<.0001$; and $F(6,152) = 7.86$, $p<.0001$; respectively].

Access to sweet food increased food efficiency ratings [$F(1,77) = 5.47$, $p<.03$]. Prior nicotine administration (i.e., cessation of nicotine) also increased food efficiency [$F(1,77) = 24.73$, $p<.0001$]. In contrast, exercise reduced food

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efficiency [$F(2,77) = 29.13$, $p<.0001$]. Post hoc Scheffe's tests indicated that the sedentary, low, and high exercise groups all differed from each other.

Because the univariate ANOVA and post hoc test results for plasma insulin and fat index variables are identical to the corresponding results in the first MANOVA and were presented, they will not be presented here.

In summary, cessation of nicotine administration increased food efficiency and decreased proportion of body fat. The decreased proportion of body fat in nicotine-treated animals may have been due to nicotine administration, and the fact that two-thirds of these animals were exercised during the five-week cessation period. Exercise reduced food efficiency, plasma insulin levels, and proportion of body fat. Access to sweet increased food efficiency, plasma insulin levels, and proportion of body fat. (See Table 1 for summary of MANOVA analyses.)

These findings suggest that nicotine cessation increases the tendency to gain weight, regardless of caloric consumption, as does access to sweet food. Exercise reduces the tendency to gain weight independently of its effects on caloric consumption. Because access to sweet food increases plasma insulin levels, and exercise decreases plasma insulin levels, insulin may mediate weight changes induced by these manipulations.

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Multiple Regressions - cessation phase

The third type of analysis performed was multiple regression analysis (Cohen & Cohen, 1983; Harris, 1985). Six separate multiple regressions were used to determine the amount of variance accounted for by the independent variables and the hypothesized mechanism of weight change, plasma insulin levels, for each of the following dependent variables: weight change between drug administration and cessation, fat index, daily caloric consumption, sweet food consumption, bland food consumption, and food efficiency. Stepwise regression procedures were used in these analyses. Insulin was entered in the regression equation first because it was the hypothesized mechanism accounting for the weight and food consumption changes due to the diet, drug, and exercise manipulations. Any diet, drug, and exercise variables that made a statistically significant contribution to the predictive capacity of the regression equation once the variance due to insulin was removed were then entered in the regression equation. The three independent variables were entered in the equation using "dummy-variable coding" (Cohen & Cohen, 1983). Exercise was assumed to be an ordinal level variable, rather than nominal.

Although the proportion of variance due to the drug manipulation was of interest, the drug condition was not entered in the regression equation first because, based on the results of the MANOVAs, the drug manipulation did not affect plasma insulin levels, and was independent of the other two

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manipulated variables entered in the equation. Nicotine cessation did not increase plasma insulin levels as hypothesized. However, plasma insulin levels were measured after five weeks of cessation in this study. Plasma insulin levels may have been elevated early in the cessation phase and then returned to control levels within five weeks of cessation. Therefore, order of entry would not affect the amount of variance accounted for by the drug manipulation.

Cessation body weight gain. Plasma insulin levels were linearly related to cessation weight gain [$F(1,87) = 17.53$, $p<.0001$] and predicted 16.8% of the variance in cessation weight gain. When both insulin and exercise were entered as predictors, this set of predictors was linearly related to cessation weight gain and predicted 41.9% of the variance. Exercise alone predicted 34.9% of the variance. Therefore, exercise and insulin share approximately 10% of the variance (i.e., insulin (16.8%) + exercise (34.9%) = 51.7%; 51.7% - exercise & drug (41.9%) = 9.8% overlap of variance). When drug condition was added to the set of predictors, the set of predictors was linearly related to cessation weight gain [$F(3,85) = 26.20$, $p<.0001$], and 48.0% of the variance was predicted, indicating that the drug manipulation predicted approximately 6% additional variance in cessation weight gain. As with the MANOVA analysis, diet was not linearly related to cessation weight gain.

In summary, plasma insulin levels, exercise, and drug manipulation are linearly related to cessation weight gain,

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and the exercise manipulation affects cessation weight gain in addition to any of its effects on plasma insulin levels.

Fat index. Plasma insulin levels were linearly related to the fat index [$F(1,87) = 59.51$, $p<.0001$] and predicted 40.6% of the variance. When the drug manipulation was added to the regression equation, insulin levels and drug condition were linearly related to the fat index [$F(2,86) = 37.17$, $p<.0001$] and predicted 46.4% of the variance. Adding the exercise manipulation to the regression increased the amount of variance predicted to 50.4%. A linear relationship between the three predictor variables and the fat index was statistically significant [$F(3,85) = 28.82$, $p<.0001$]. Adding diet to the regression equation did not significantly contribute to the predictive capacity of the equation.

Daily caloric consumption. The diet manipulation was linearly related to daily caloric consumption [$F(1,87) = 8.70$, $p<.005$] and predicted 9.1% of the variance. Adding the exercise manipulation to the regression equation increased the variance predicted to 12.7% and resulted in a linear relationship between the predictors and caloric consumption [$F(2,86) = 6.25$, $p<.003$]. Plasma insulin levels 5 weeks after cessation of nicotine administration and nicotine cessation did not contribute significantly to the predictive capacity of the regression equation.

Sweet food consumption. Plasma insulin levels and the exercise and drug manipulations were not linearly related to sweet food consumption individually, or as a set, and

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accounted for only 9.9% of the variance in sweet food consumption as a set. Although, when all three variables were entered in the regression equation, the contribution by exercise approached significance [$F(1,42) = 3.47$, $p<.07$].

Bland food consumption. Insulin levels were not linearly related to bland food consumption. Diet was linearly related to bland food consumption [$F(1,87) = 1068.38$, $p<.0001$] and accounted for 92.5% of the variance in bland food consumption. Adding the drug manipulation to the regression equation increased the variance predicted to 93.0%, and resulted in a linear relationship between the two predictors and bland food consumption [$F(2,86) = 567.99$, $p<.0001$]. Exercise did not contribute significantly to the predictive capacity of the regression equation.

Food efficiency. Plasma insulin levels were linearly related to food efficiency [$F(1,87) = 18.95$, $p<.0001$], and predicted 17.9% of the variance. Adding the independent predictor variables that made a statistically significant contribution to the amount of variance predicted (drug and exercise) by the regression equation also produced a linear relationship between the set of predictors and food efficiency [$F(3,85) = 35.01$, $p<.0001$] and predicted 55.3% of the variance.

In summary, plasma insulin levels were statistically significant predictors in regression equations for the following dependent variables: cessation weight gain, fat index, and food efficiency. The diet manipulation was not a

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statistically significant predictor for any of these three dependent variables once variance due to plasma insulin levels was removed, suggesting that the effect of the diet manipulation on fat index values was largely due to the diet manipulation's effects on plasma insulin levels. Diet was a statistically significant predictor for daily caloric consumption and bland food consumption. The drug manipulation significantly increased the predictive capacity of the regression equation once variance due to insulin levels was removed in the following dependent variables: cessation weight gain, fat index, and food efficiency. The drug manipulation was also a significant predictor for bland food consumption. Exercise significantly increased the predictive capacity of the regression equation once variance due to plasma insulin levels was removed in the following dependent variables: cessation weight gain, fat index, and food efficiency. It also was a significant predictor for daily caloric consumption. (See Table 2 for summary of regression analyses.)

Citrate Synthase

Because citrate synthase was not hypothesized to be related to the changes in body weight and was being used as an index of physical training, it was analysed separately from the other dependent variables. A three-way ANOVA (2 x 2 x 3) was used to assess the effects of diet, drug, and exercise manipulations on citrate synthase levels in muscle tissue.

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Exercise increased citrate synthase activity in gastrocnemious muscle tissue [$F(1,7) = 3.48$, $p<.04$]. Scheffe's post hoc tests indicated that only the sedentary and high exercise groups differed. All other independent variables and interactions did not affect citrate synthase activity.

Physical Activity

The number of rats used in monitoring general physical activity in the photocell chambers was too small for this variable to be included in any of the multivariate analyses. Because of the small number of animals per group (two), some of the groups were collapsed for each analysis. A t-test was performed on baseline phase horizontal physical activity measures to determine if diet affected physical activity. Rats with access to bland food averaged 1,398 horizontal units of activity per hour, and rats with access to both bland and sweet food averaged 1,683 units per hour. A t-test was also performed on change scores between baseline and drug administration physical activity measures to determine if nicotine administration affected physical activity. Rats that received saline averaged 2,455 units of activity per hour, and rats that received nicotine averaged 1,978 units per hour. Two ANCOVAs were used to determine if the exercise manipulation affected physical activity outside of the exercise period (cessation phase monitoring during the second and fifth week of exercise with baseline physical activity as a covariate). During the second week of exercise, rats in the

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sedentary, low exercise, and high exercise groups averaged 1,375; 2,123; and 1,444 units of activity per hour, respectively. During the fifth week of exercise, rats in the sedentary, low exercise, and high exercise groups averaged 1,007; 1,021; and 1,301 units of activity per hour, respectively.

Diet, drug, and exercise manipulations did not affect physical activity measures. Therefore, spontaneous physical activity was not responsible for the effects of either nicotine or physical exercise on body weight. Also, the exercise manipulation did not reduce physical activity during the active phase (dark period) of the rats by increasing sleep, or by simply reducing physical activity.

Hypotheses Summary

Baseline Period: Body Weight and Food Intake.

1) Rats with access to sweet food had similar body weights to those that had access to only bland food.

2) Rats with access to sweet food consumed less bland food than rats having only bland food.

3) Access to sweet food increased total caloric consumption during the baseline phase.

During Drug Administration: Body Weight.

1) Although rats receiving saline weighed more than rats receiving nicotine, access to sweet food did not increase body weight.

2) Rats receiving nicotine that also had access to sweet food had the lowest body weights during the drug administration phase.

3) Rats receiving nicotine that had access to only bland food had body weights that were intermediate to rats receiving nicotine with access to sweet food and rats receiving saline, regardless of diet condition.

During Drug Administration: Food Intake.

1) Rats with access to only bland food that were receiving nicotine consumed fewer calories than rats with access to sweet food that were receiving saline.

2) Although bland food consumption increased in rats receiving nicotine with access to sweet food, bland food consumption in these rats remained well below bland food consumption values for all rats with access to only bland food.

3) Rats with access to sweet food that were receiving saline had the lowest bland food consumption values.

4) Rats receiving nicotine consumed less sweet food than rats receiving saline.

5) Although rats receiving saline consumed the largest number of kilocalories, access to sweet food did not increase caloric consumption in these rats.

Drug Cessation Period: Body Weight.

1) Sedentary rats with access to only bland food that had received saline had the highest body weights.

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2) Exercising rats with access to sweet food that had received nicotine had the lowest body weights during the cessation phase.

3) Exercise reduced body weight gains within each of the drug and diet conditions.

4) Access to sweet food slighly reduced cessation body weight.

5) Rats that had received nicotine during the drug administration period had lower body weights after cessation than rats that had received saline within each diet and exercise group, except for the sedentary rats with access to sweet food. Sedentary rats with access to sweet food had similar body weights regardless of the drug treatment they received.

Drug Cessation Period: Food Intake.

1) Rats with access to bland food had similar caloric consumption values, except exercising rats had slightly lower caloric consumption values.

2) Rats with access to sweet food consumed similar amounts of bland food.

3) Sedentary rats that had received nicotine consumed the most sweet food during the cessation phase.

4) Exercise tended to reduce sweet food consumption, but this effect did not reach statistical significance.

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5) Rats that had received nicotine during the drug administration period consumed more sweet food than rats that received saline within the sedentary and low exercise groups, but not in the high exercise group. However, this effect also did not reach statistical significance.

6) Sedentary rats that had received saline had the highest caloric consumption.

7) Exercise reduced caloric consumption.

8) Prior nicotine administration did not increase caloric intake relative to the saline controls during the drug cessation phase.

9) Access to sweet food only increased caloric intake during the baseline phase, and slightly reduced caloric intake during the drug administration and cessation phases.

Drug Cessation: Physiological Measures.

1) Rats with access to sweet food had higher plasma insulin levels than rats with access to only bland food within each drug and exercise group, except for the high exercise groups.

2) Prior nicotine administration did not affect plasma insulin levels when measured five weeks after cessation of nicotine administration.

3) Exercising rats had lower plasma insulin levels than sedentary rats within each diet and exercise group.

4) Exercising rats had higher muscle tissue levels of citrate synthase than sedentary rats.

5) Access to sweet food increased proportion of body fat.

6) Rats that had received nicotine had fat pads that contributed a lower percentage of total body weight than rats that had received saline.

7) Exercising rats had fat pads contributing a lower percentage of their total body weight than sedentary rats within each drug and diet group.

DISCUSSION

This section begins with a brief summary of the findings of this study and an application of these results to human smokers who want to quit smoking without gaining weight. Next, the implications of the obtained results for theories concerning mechanisms of the weight changes during nicotine administration and cessation, and the attenuation of cessation weight gain by exercise are discussed. Finally, this section concludes with a brief discussion of future research concerning nicotine cessation-induced weight gain.

Nicotine or saline was continuously administered for two weeks via osmotic minipumps to rats with access to either bland food only or bland and sweet food. Following the removal of the minipumps, one third of the rats remained sedentary, one third swam for periods up to 30 minutes per day, and one third swam for periods up to 60 minutes per day. Plasma insulin levels and fat pad weights were measured at the end of the five-week cessation phase.

Exercise greatly reduced nicotine cessation-induced weight gain and proportion of body fat. In addition, a combination of exercise (corresponding to the high exercise group in this study) and access to sweet food is more effective in preventing this weight and fat gain than light levels of exercise with or without access to sweet food, or than restricted access to sweet food without exercise. Exercise and restricting access to sweet food also reduced plasma insulin levels.

Practical Implications for Future Ex-smokers

Based on the comparability of findings in previous animal and human studies of nicotine and body weight, it is likely that the results of the present rat study will generalize to human smokers. Therefore, it seems that the optimal way for ex-smokers to avoid weight gain is to exercise at moderate levels. However, because cigarette smoking is associated with heart disease, some smokers may not be able to safely exercise at moderate levels. The next best approach to prevent cessation weight gain would be to exercise lightly and restrict access to sweet food. Although restricting access to sweet food probably would not reduce weight gain, it would reduce proportion of body fat. For those smokers who wish to prevent cessation weight gain and cannot, or will not, increase their physical activity level, restricting access to sweet food may be of some help.

A word of caution concerning access to sweet food needs to be added here. In this study, sweet food only differed from the bland food by sugar (carbohydrate) composition. Sweet foods consumed by humans frequently have a high fat content, which markedly increases the caloric density of the food. Therefore, the results of the present study may only hold for low-fat sweet food.

Although this study clearly demonstrated that exercise attenuated nicotine cessation weight gain in rats, studies of the effects of similar diet and exercise manipulations on human smokers who quit smoking (including men and women) need

to be conducted to ensure that these findings can be applied to human smokers who quit smoking. Studies of human smokers may be particularly important because rats and humans have different growth patterns. Rats continue to grow and gain weight throughout their life span. Therefore, the present study examined effects of nicotine, exercise, and food intake in subjects that naturally were gaining weight. In contrast, humans do not gain body weight to this same extent throughout their life span, although it is common to gain some weight with age.

Theoretical Implications for Mechanisms

The findings of this study suggest possible mechanisms for the reduction of weight gain by nicotine administration, increase in weight gain by nicotine cessation, and the attenuation of nicotine cessation weight gain by diet and exercise manipulations. The reduction of weight gain results from reduction in energy intake, or absorption, and from increases in energy expenditure. Nicotine administration reduces energy intake if the diet includes sweet food. Therefore, reduction in energy intake contributes to nicotine-induced reduction of weight gain. Results of the food efficiency analyses indicate that other factors also are involved in nicotine's reduction of weight gain. Because food efficiency reflects weight gain per gram of original body weight per kilocalorie consumed, the tendency to gain or lose weight is independent of food intake. Nicotine administration reduced food efficiency, indicating that reduction in energy

absorption or increases in energy expenditure are also involved in nicotine's influence on body weight.

Nicotine also may reduce rate of weight gain by increasing energy expenditure during dietary-induced thermogenesis (or the thermic effect of food). Insulin administration increases thermogenesis, particularly when administered in conjunction with glucose, if hypoglycemia is not produced (Rothwell and Stock, 1988). Obese humans, who frequently have higher insulin resistance than lean individuals, require larger amounts of insulin per kilogram of body weight than lean humans to produce similar rates of glucose uptake and thermogenesis following glucose administration (Ravussin, Acheson, Vernet, Danforth, & Jequier, 1985). Also, the administration of ciglitazone, an oral hypoglycemic agent, reduces energy efficiency and increases thermogenesis (Rothwell, Stock, and Tedstone, 1987). These findings suggest that increasing insulin sensitivity, particularly in the presence of increased plasma glucose levels, may increase thermogenesis and reduce food efficiency.

Because chronic nicotine administration reduces plasma insulin levels (Grunberg, Popp et al., 1988), insulin sensitivity may also increase, and thereby increase dietary-induced thermogenesis (DIT). In support of this hypothesis, Robinson and York (1986) reported that DIT was 35% higher in smokers than in non-smokers. This hypothesis could also explain why nicotine-treated animals in the present study gained less weight when they had access to sweet food than

when they only had access to bland food. That is, chronic nicotine administration may have suppressed plasma insulin levels, increased insulin sensitivity and DIT, and thereby reduced food efficiency.

At first glance, the fact that insulin levels did not differ between saline and nicotine-treated animals and food efficiency was higher in nicotine-treated rats might suggest that insulin and DIT were not involved in the nicotine cessation-induced increase in food efficiency. However, food efficiency is based on weight changes and food consumption during the entire cessation phase. Plasma insulin levels have been reported to increase beyond saline control levels one week after nicotine cessation (Grunberg, Raygada et al., 1988). However, insulin levels may have increased and then returned to control levels by the fifth week of nicotine cessation. Therefore, changes in plasma insulin levels and DIT may have been involved in the higher food efficiency rates and increased weight gain during nicotine cessation, but prior to five weeks after cessation of nicotine administration.

Consistent with this hypothesis, exercise reduced plasma insulin levels and food efficiency. Exercise increases insulin sensitivity (Soman, Veikko, Derbert, Felig, & DeFronzo, 1979) and thermogenesis in the presence of glucose (Balon, Zorzano, Goodman, & Ruderman, 1986). Access to sweet food also reduced weight gain in exercising rats that had received nicotine, but not in sedentary rats or rats that had received saline. Because access to sweet food increased food

efficiency in all rats except those in the nicotine and saline drug conditions in the high exercise groups, it seems likely that access to sweet food reduces body weight in exercising rats by reducing caloric intake. However, exercising the nicotine-treated rats may have maintained a nicotine-induced increase in insulin sensitivity and DIT, while exercising saline controls may have produced these same effects. Exercise may also have reduced cessation weight gain by increasing energy expenditure based on the caloric expenditure of the exercise and any changes in resting metabolic rate that may have occurred, and these increases in energy expenditure may have masked any diet-induced effects on DIT.

In addition, exercise reduced caloric consumption, which also may have contributed to the attenuation of weight gain during nicotine cessation. Central mechanisms, particularly central serotonin levels, may induce the reduction in caloric intake. Central or peripheral administration of serotonin or drugs that enhance or mimic serotonin activity reduce food consumption (Blundell, 1984), particularly carbohydrate consumption (Blundell & McArthur, 1979; Wurtman & Wurtman, 1979, 1984). If chronic nicotine administration increases central serotonin levels, it could reduce sweet food consumption via this central mechanism. Balfour and co-workers (1986) reported that nicotine administration reduced hippocampus serotonin levels, but did not affect hypothalamic serotonin levels. However, nicotine administration consisted of daily injections for only 6 days.

Therefore it is possible that chronic nicotine administration may affect hypothalamic serotonin levels. In addition, the authors did not report whether or not the brain tissue was collected from fasting animals. Because carbohydrate intake can affect central serotonin levels, food intake may prevent the detection of differences in serotonin and needs to be controlled in future studies. A review of literature in this area concluded that studies of nicotine's effects on serotonin do not provide firm conlusions on the effect of nicotine on serotonergic neurotransmission (USDHHS, 1988). Although exercise did not specifically reduce sweet food consumption (as did nicotine administration), both diets were fairly high in carbohydrate content. Also, exercising rats consumed less sweet food, but the differences in consumption were not statistically significant. Therefore, the slightly lower levels of sweet food consumption in exercising rats may have contributed to the reduction in caloric consumption.

Increasing plasma insulin levels (either directly, or by providing a high carbohydrate meal) increases plasma tryptophan and brain serotonin levels (Wurtman & Wurtman, 1971). Although exercise reduces plasma insulin levels, it increases insulin sensitivity and glucose uptake, and could increase brain serotonin levels, thereby reducing caloric consumption. As nicotine administration reduces plasma insulin levels, nicotine also may increase insulin sensitivity and raise central serotonin levels. However, further research is needed to determine if: (1) nicotine administration

increases insulin sensitivity, and (2) increasing insulin sensitivity increases central serotonin levels.

In summary, exercise may prevent or attenuate nicotine cessation-induced weight gain through changes in insulin sensitivity, which may increase DIT and reduce food intake by increasing central serotonin levels. Exercise may also attenuate weight gain by increasing energy expenditure through the caloric cost of the exercise and/or increasing resting metabolic rate.

Further studies concerning mechanisms underlying nicotine-induced weight changes need to be conducted. Specifically, the effects of nicotine administration on insulin sensitivity and central serotonin levels, as well as the effects of insulin sensitivity and exercise on central serotonin levels, need to be explored. Exploration of these issues may lead to a better understanding of nicotine administration and cessation-induced body weight changes, and alternative ways to prevent nicotine cessation-induced weight gain, such as the careful administration of oral hypoglycemic agents or the administration of pharmacological agents that enhance serotonin activity, may be found.

Table 1
MANOVA SUMMARY
(cessation phase)

MANOVA 1

Dependent variables	Diet	Drug	Exercise
cessation weight gain	no change	(nic) increase	decrease
fat index	(w/sw) increase	(nic) decrease	decrease
insulin	(w/sw) increase	no change	decrease
caloric consumption	(w/sw) decrease	no change	decrease
water consumption	(w/sw) decrease	no change	no change

MANOVA 2
(only rats with access to sweet food)

Dependent variables	Drug	Exercise
cessation weight gain	(nic) increase	decrease
fat index	no change	decrease
insulin	no change	decrease
sweet food consumption	no change	no change
bland food consumption	no change	no change
water consumption	no change	no change

MANOVA 3

Dependent variables	Diet	Drug	Exercise
Food Efficiency	(w/sw) increase	(nic) increase	decrease
Insulin	(w/sw) increase	no change	decrease
Fat Index	(w/sw) increase	(nic) decrease	decrease

Table 2

REGRESSION SUMMARY (Cessation Phase)

Dependent Variables	Predictors
Cessation weight gain	insulin, drug, exercise
Fat index	insulin, drug, exercise
Food efficiency	insulin, drug, exercise
Daily caloric consumption	diet, exercise
Sweet food consumption	(none)
Bland food consumption	drug, diet

Figure 1
BODY WEIGHT ACROSS PHASES

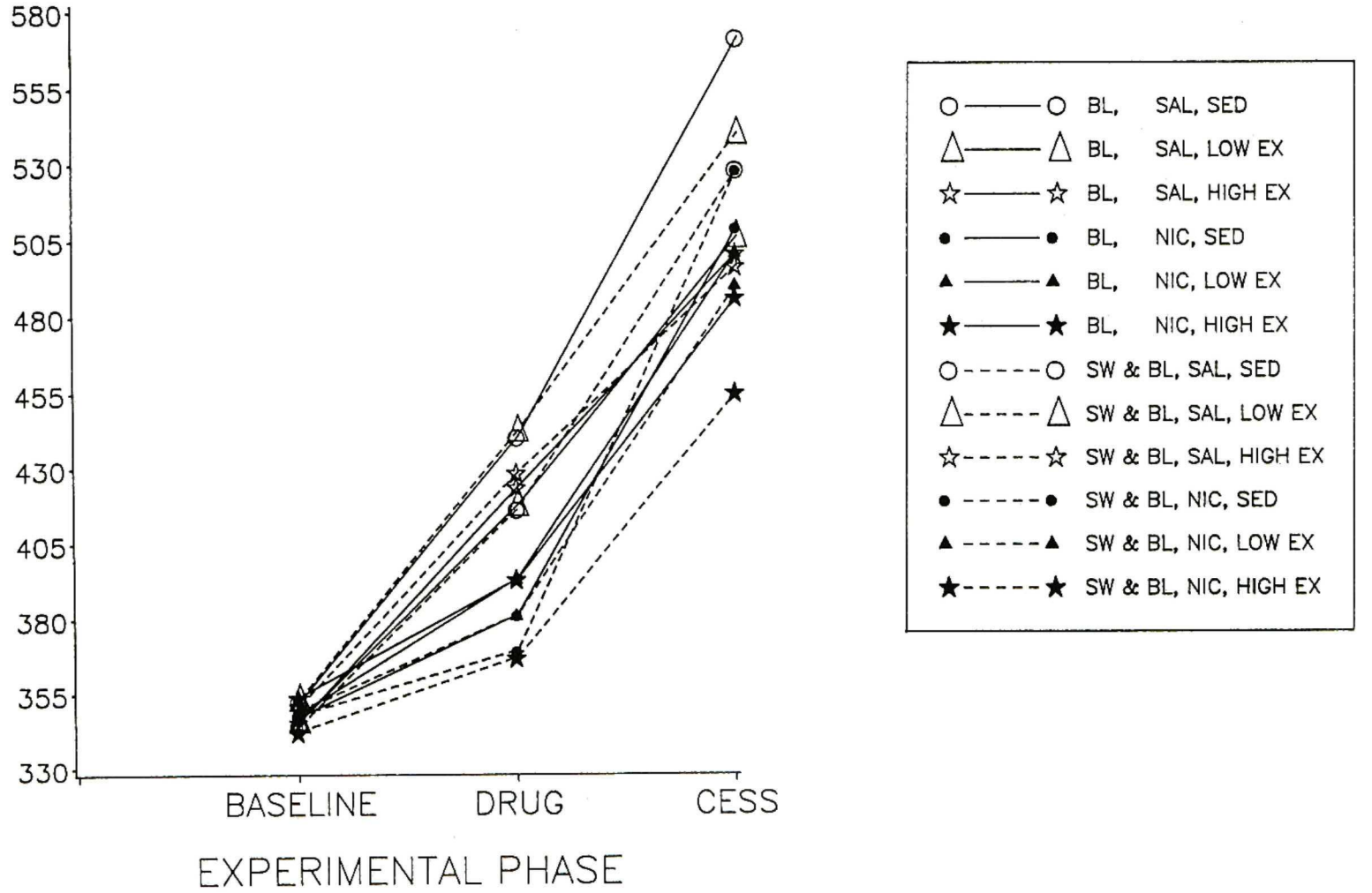


Figure 2
BODY WEIGHT ACROSS PHASES
COLLAPSED ACROSS EXERCISE TYPES

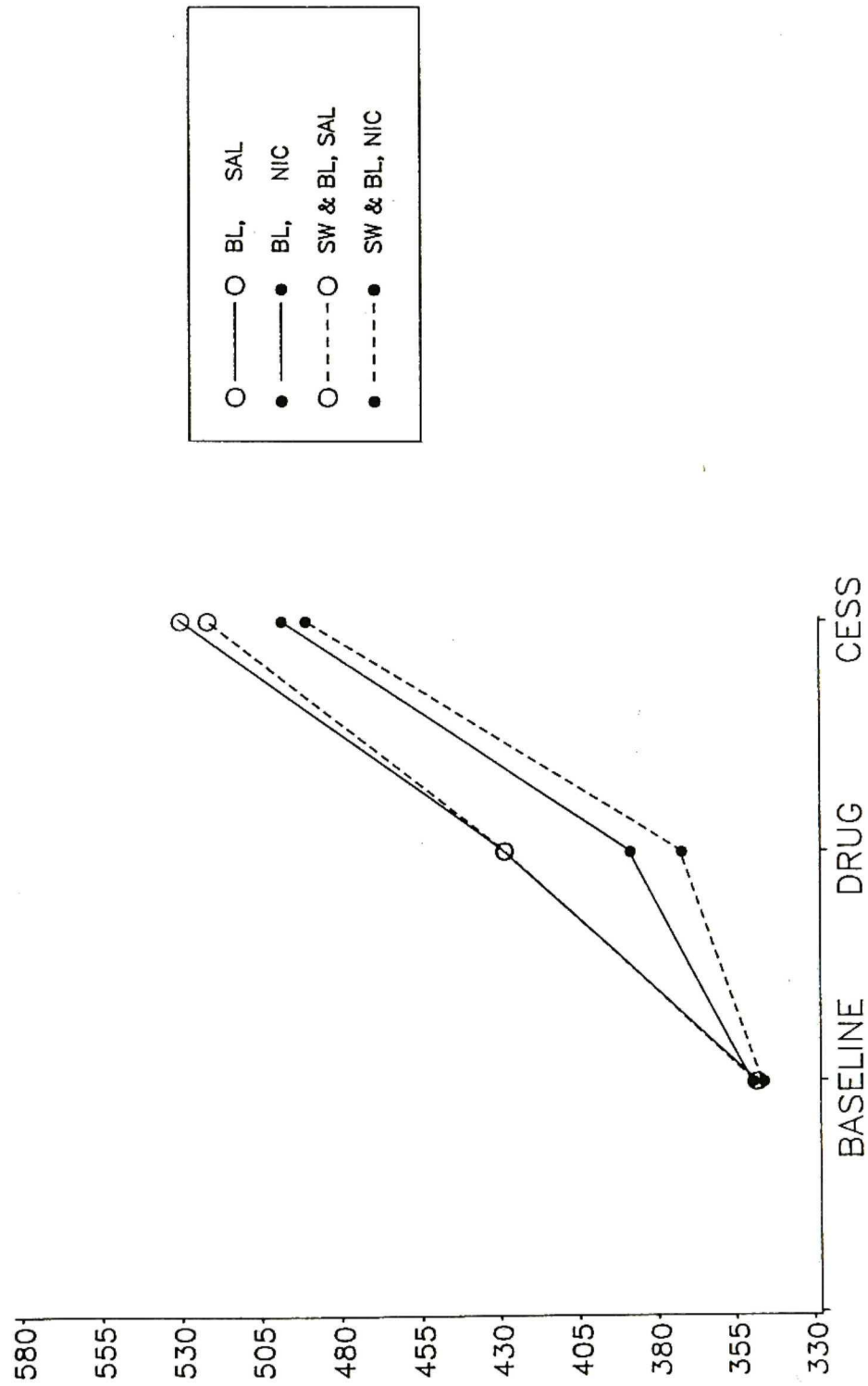


Figure 3
BODY WEIGHT ACROSS PHASES
COLLAPSED ACROSS DIET

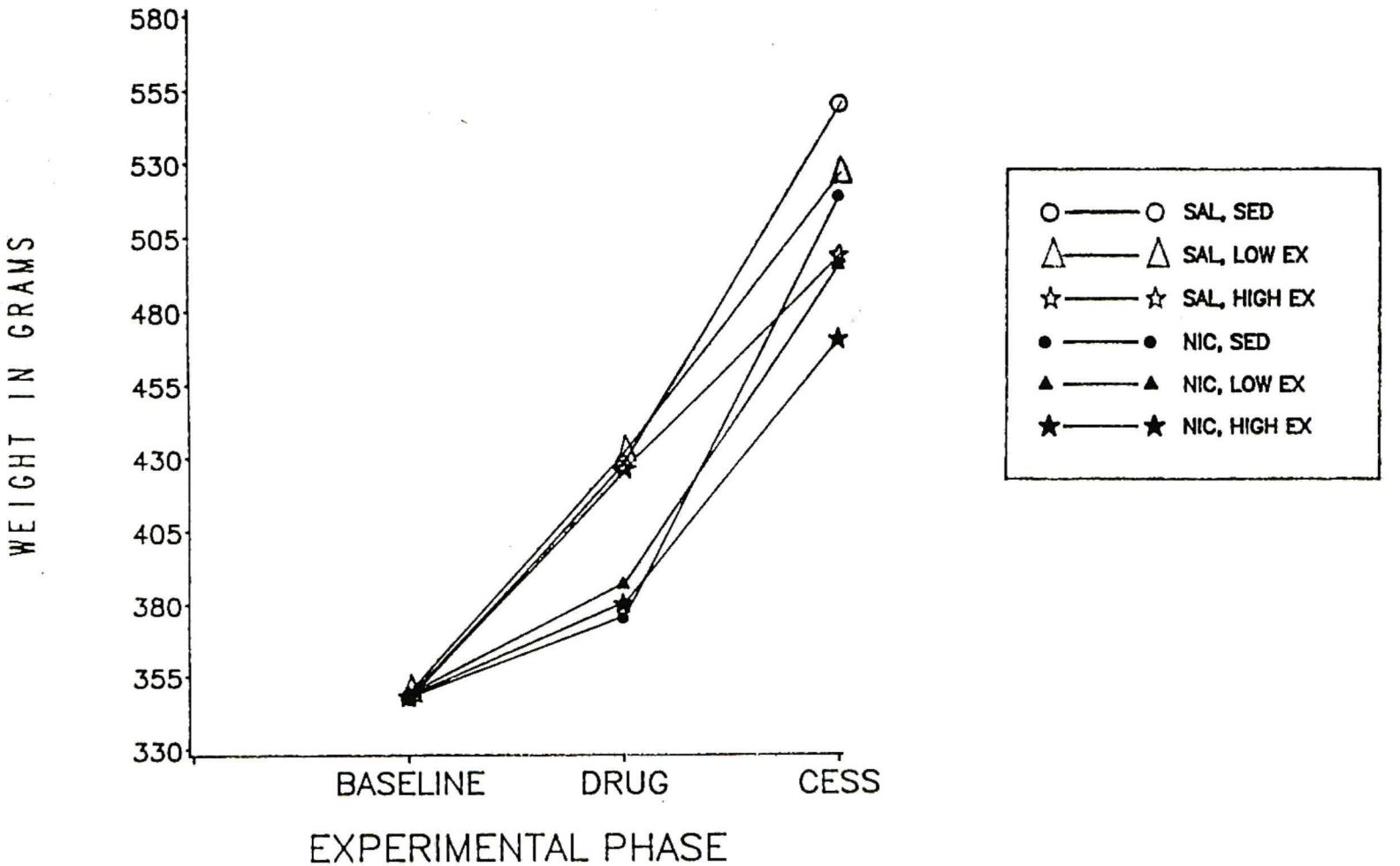


Figure 4
CALORIC CONSUMPTION ACROSS PHASES

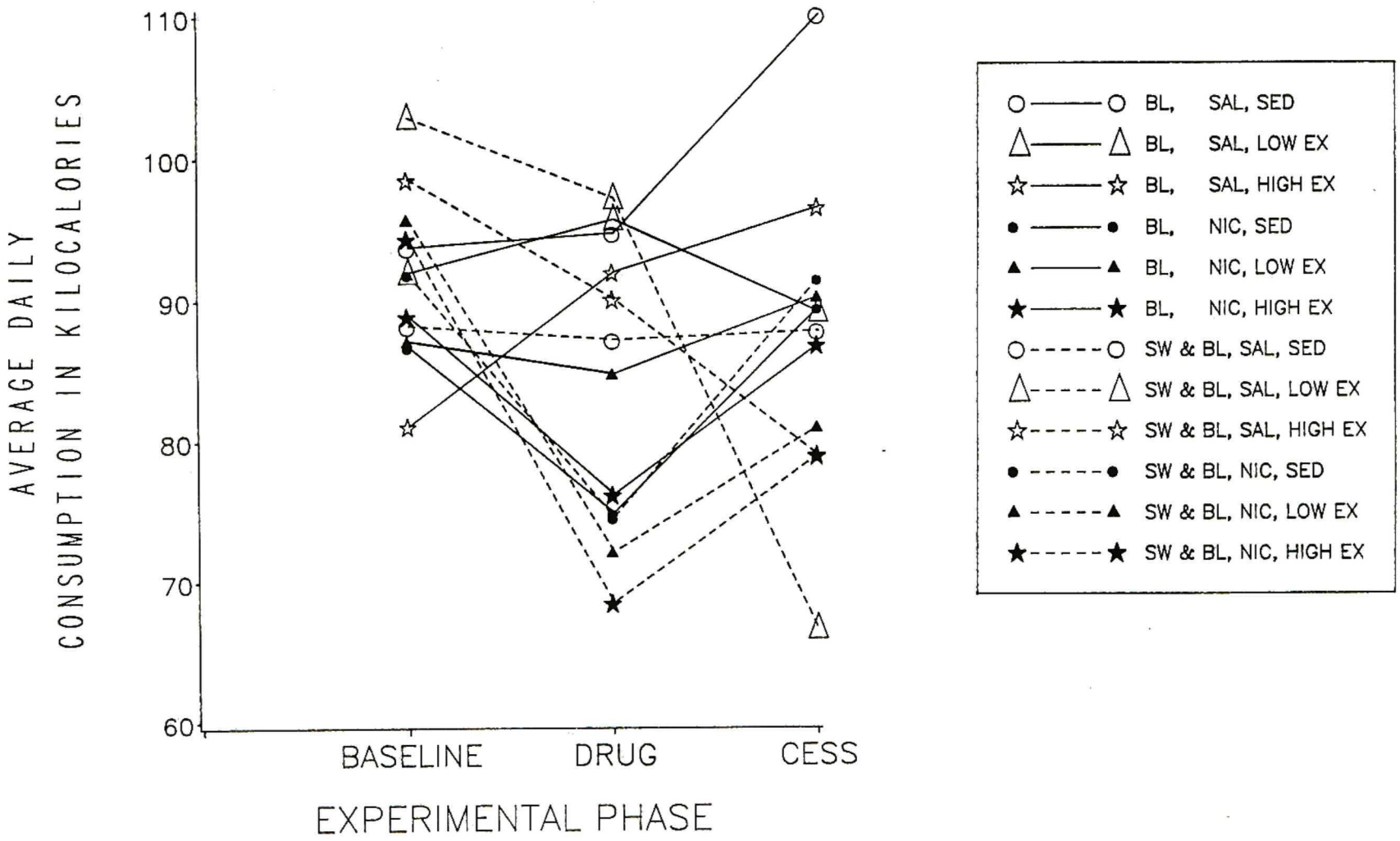


Figure 5
CALORIC CONSUMPTION ACROSS PHASES
COLLAPSED ACROSS EXERCISE TYPES

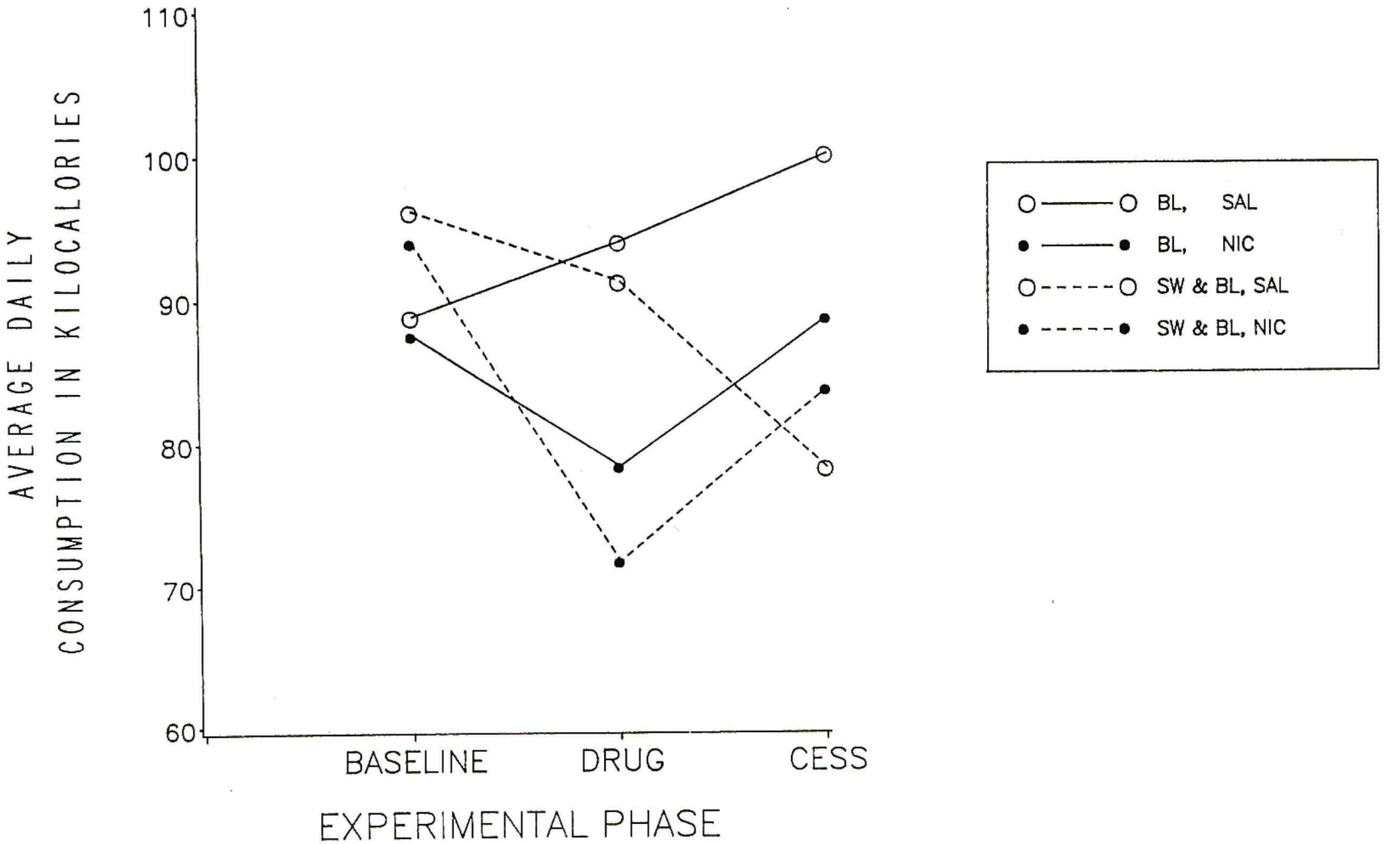


Figure 6
CALORIC CONSUMPTION ACROSS PHASES
COLLAPSED ACROSS DIET

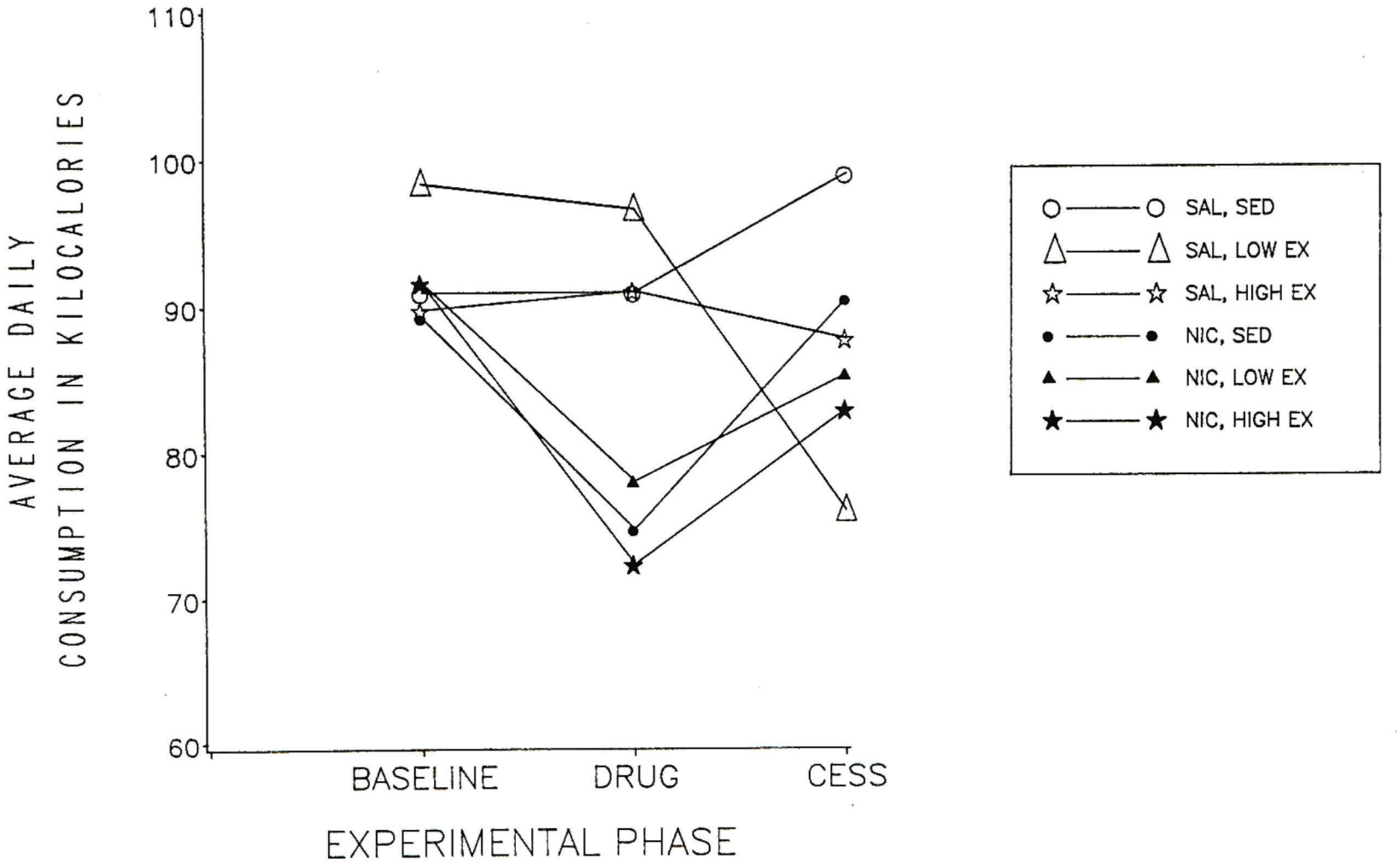


Figure 7
BLAND FOOD CONSUMPTION ACROSS PHASES

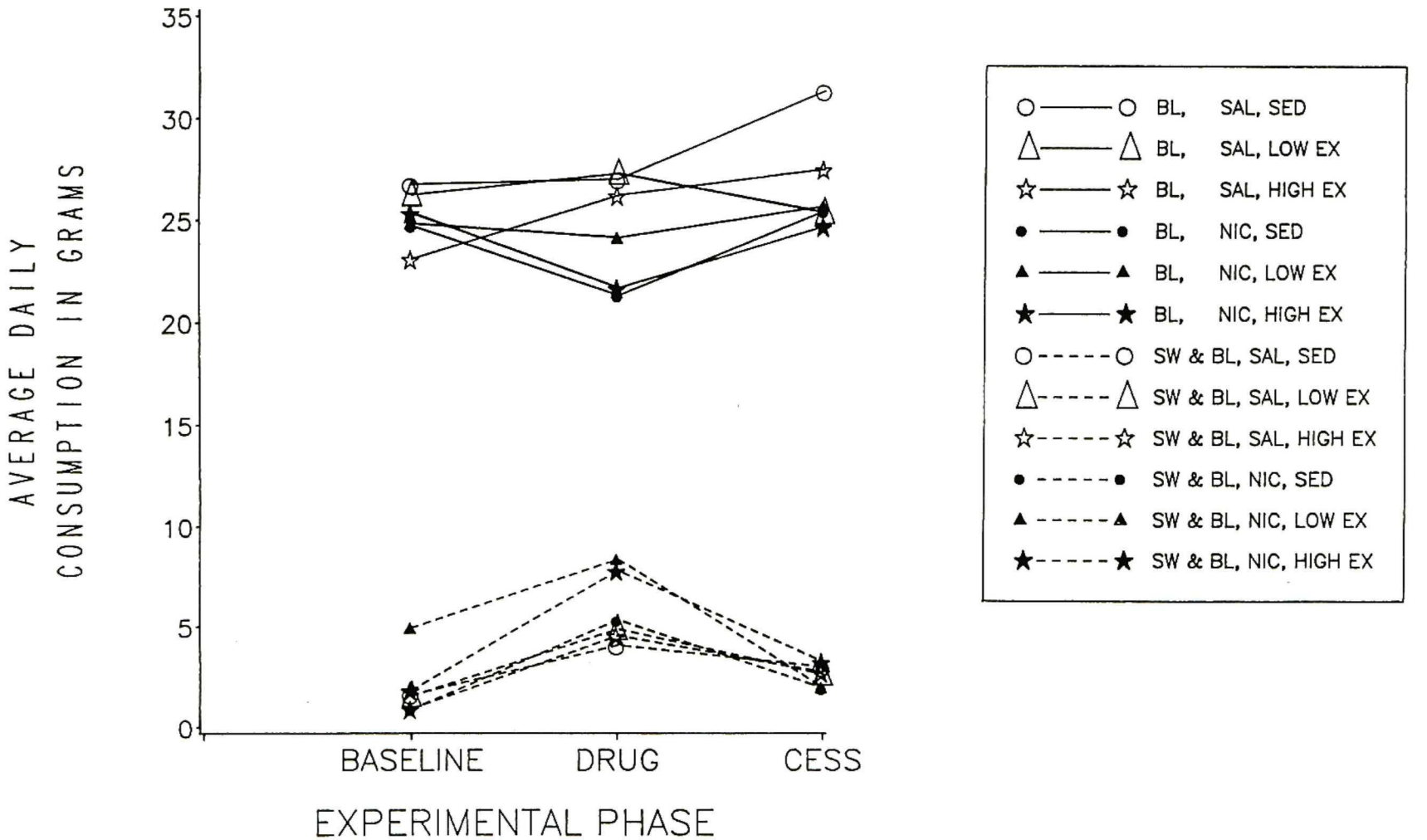


Figure 8
BLAND FOOD CONSUMPTION ACROSS PHASES
COLLAPSED ACROSS EXERCISE TYPES

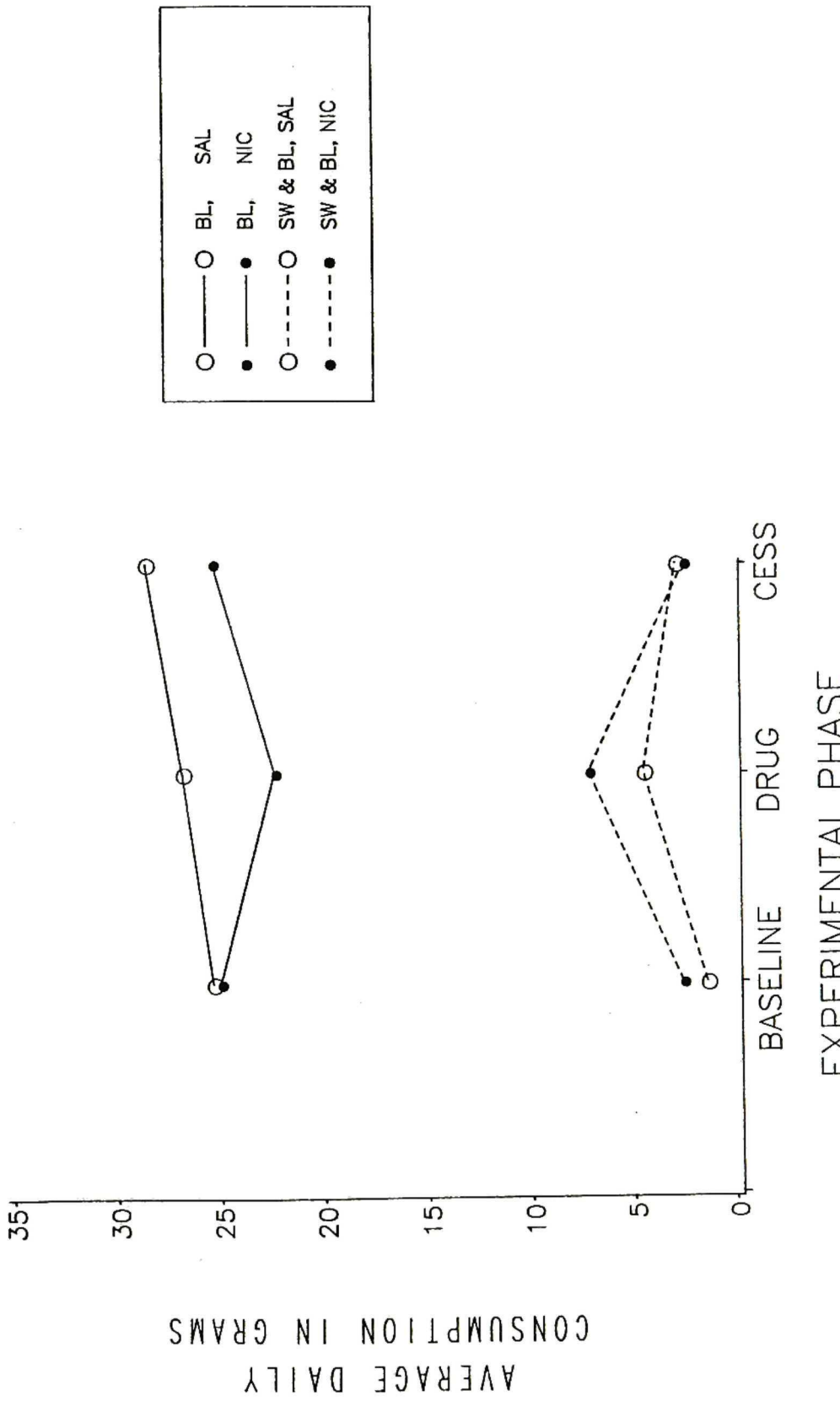


Figure 9
BLAND FOOD CONSUMPTION ACROSS PHASES
COLLAPSED ACROSS DIET

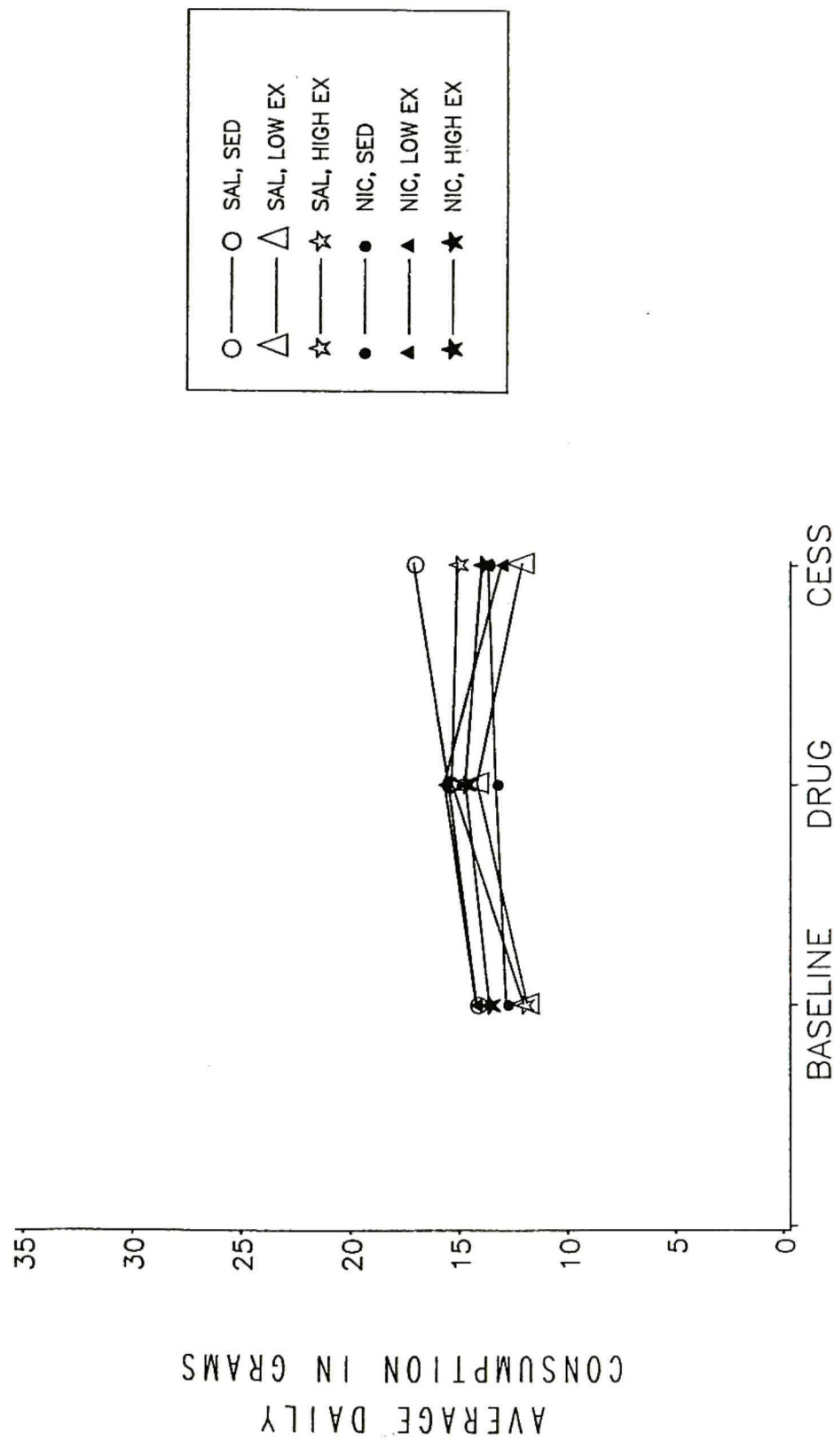


Figure 10
SWEET FOOD CONSUMPTION ACROSS PHASES

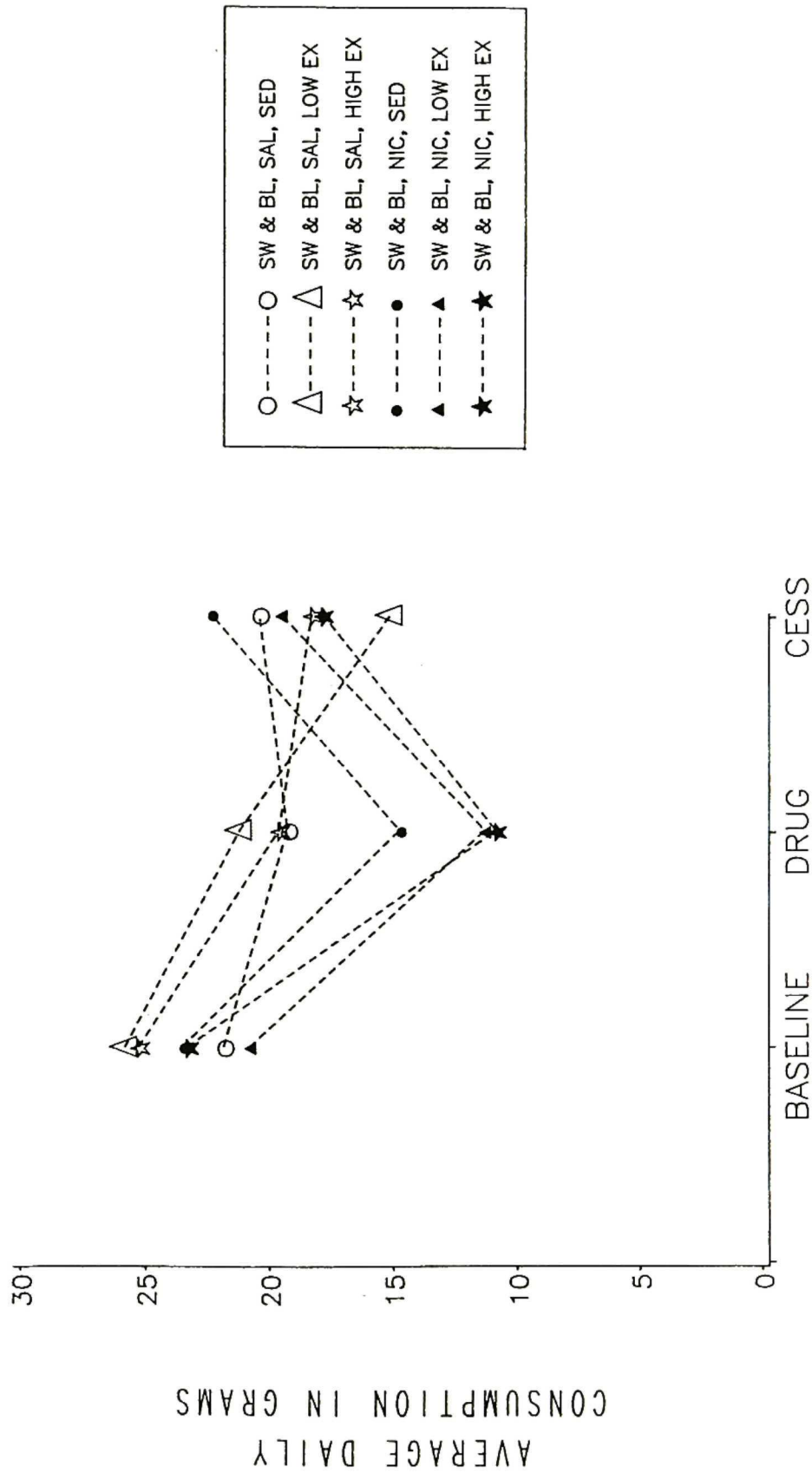


Figure 11
SWEET FOOD CONSUMPTION ACROSS PHASES
COLLAPSED ACROSS EXERCISE TYPES

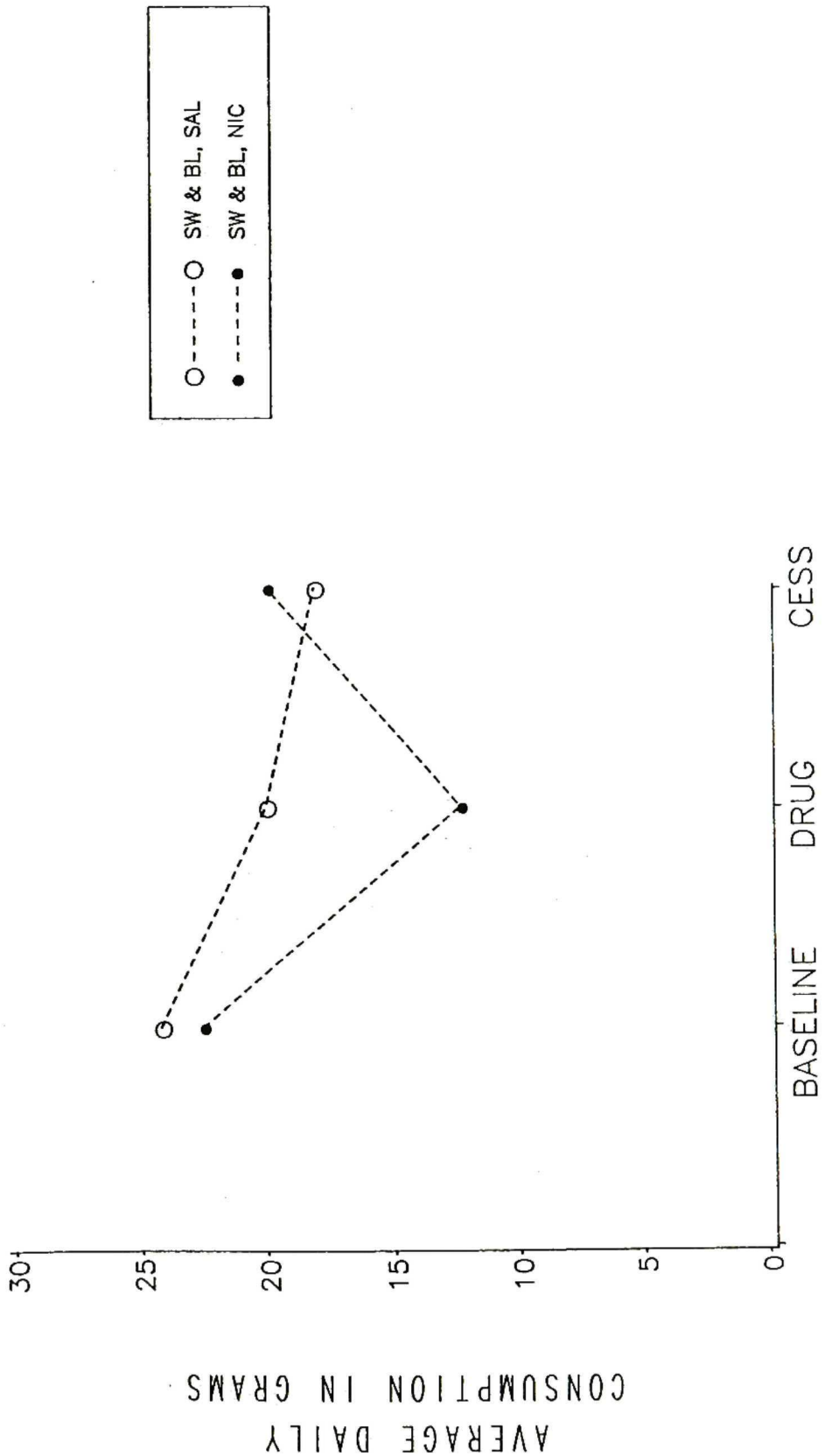


Figure 12
WATER CONSUMPTION ACROSS PHASES

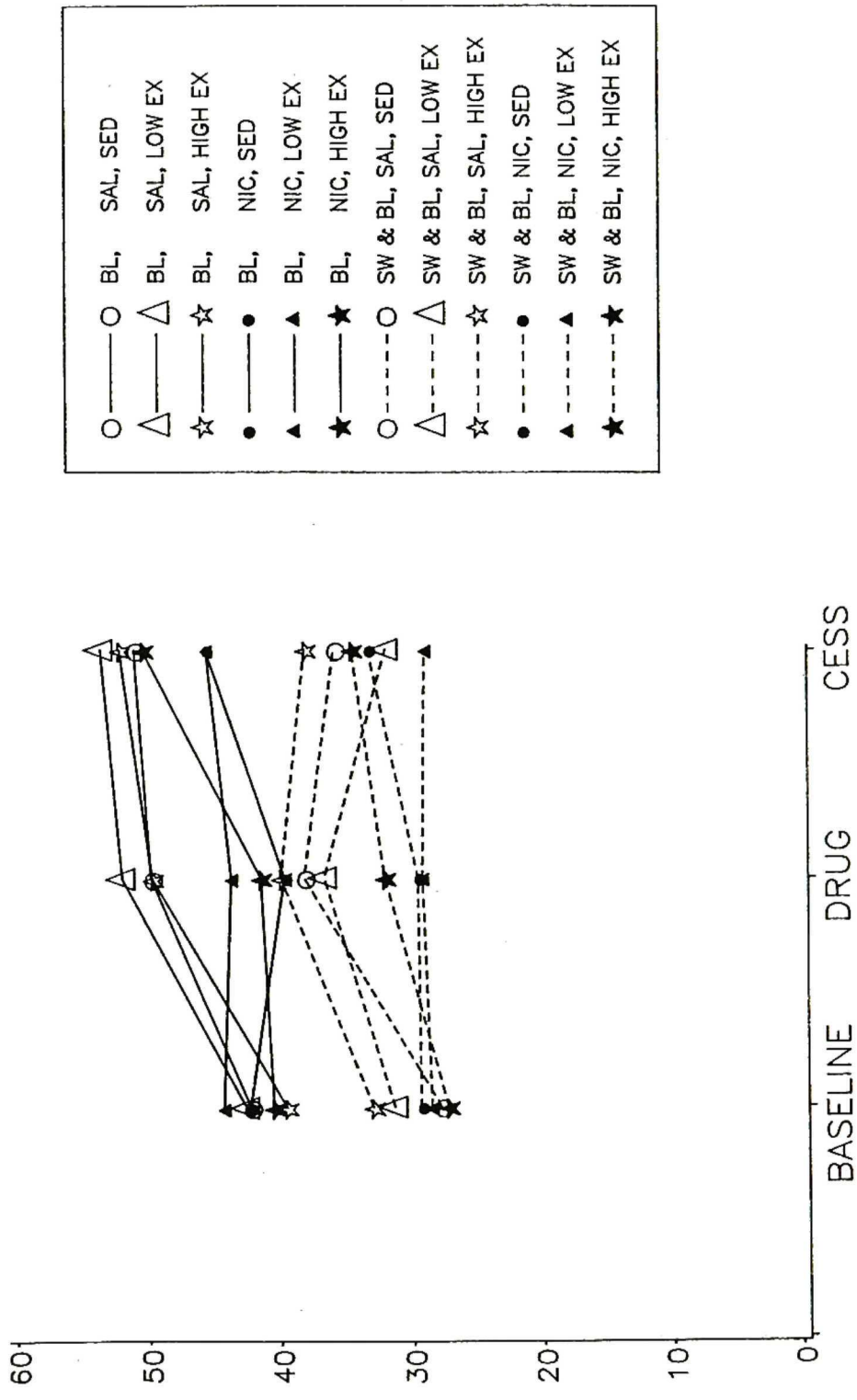


Figure 13
WATER CONSUMPTION ACROSS PHASES
COLLAPSED ACROSS EXERCISE TYPES

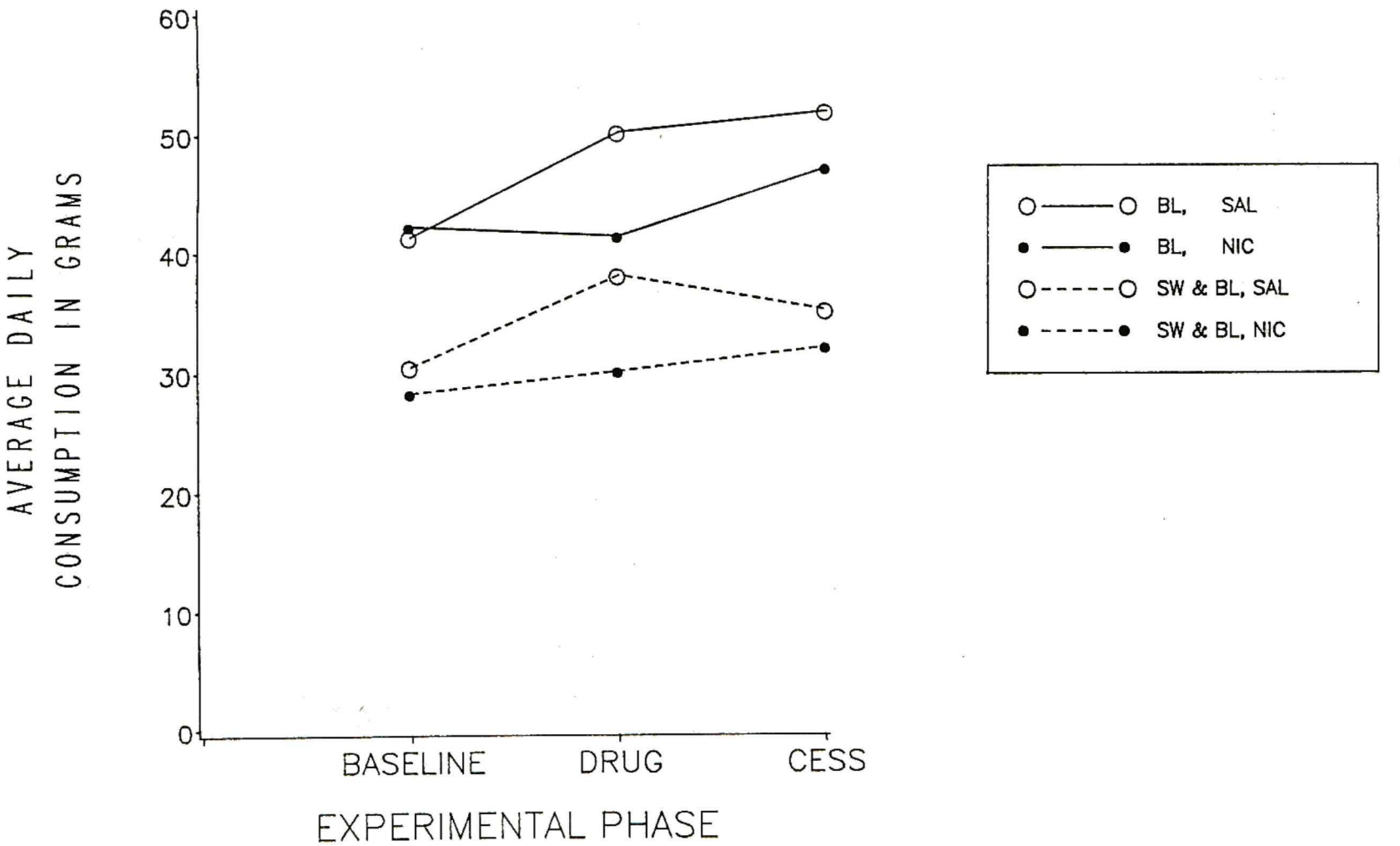


Figure 14
FOOD EFFICIENCY

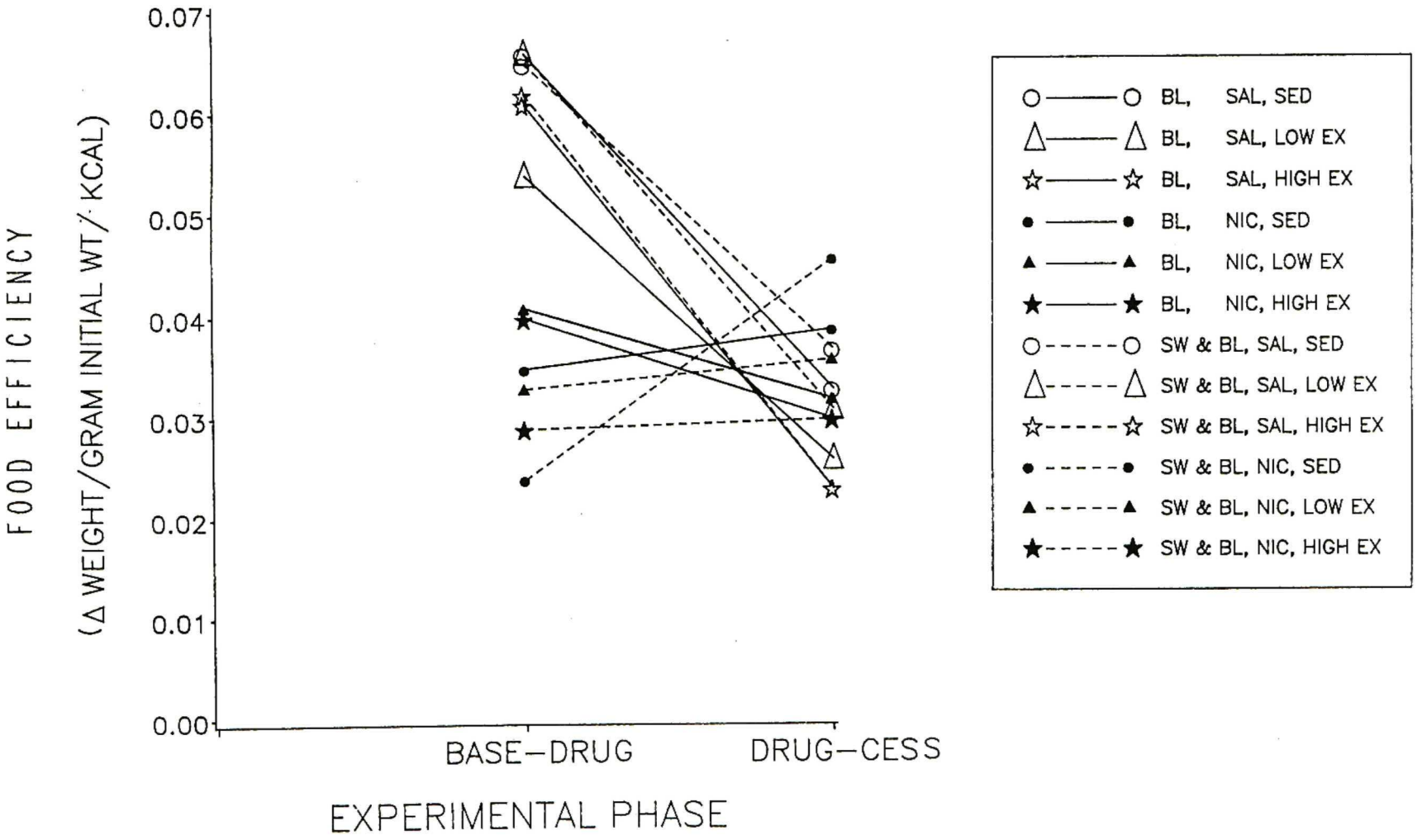


Figure 15
FOOD EFFICIENCY
COLLAPSED ACROSS EXERCISE TYPES

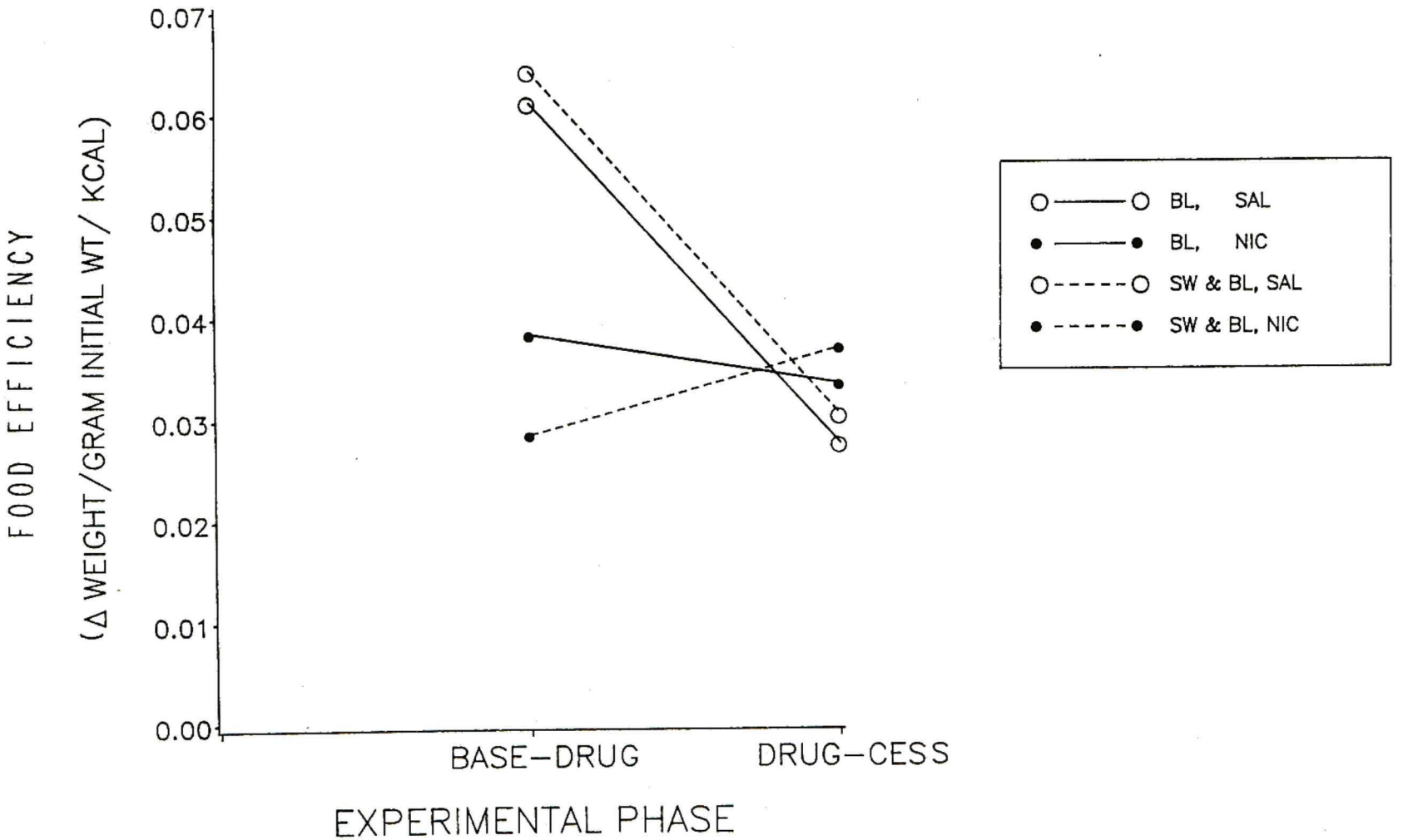


Figure 16
FOOD EFFICIENCY
COLLAPSED ACROSS DIET

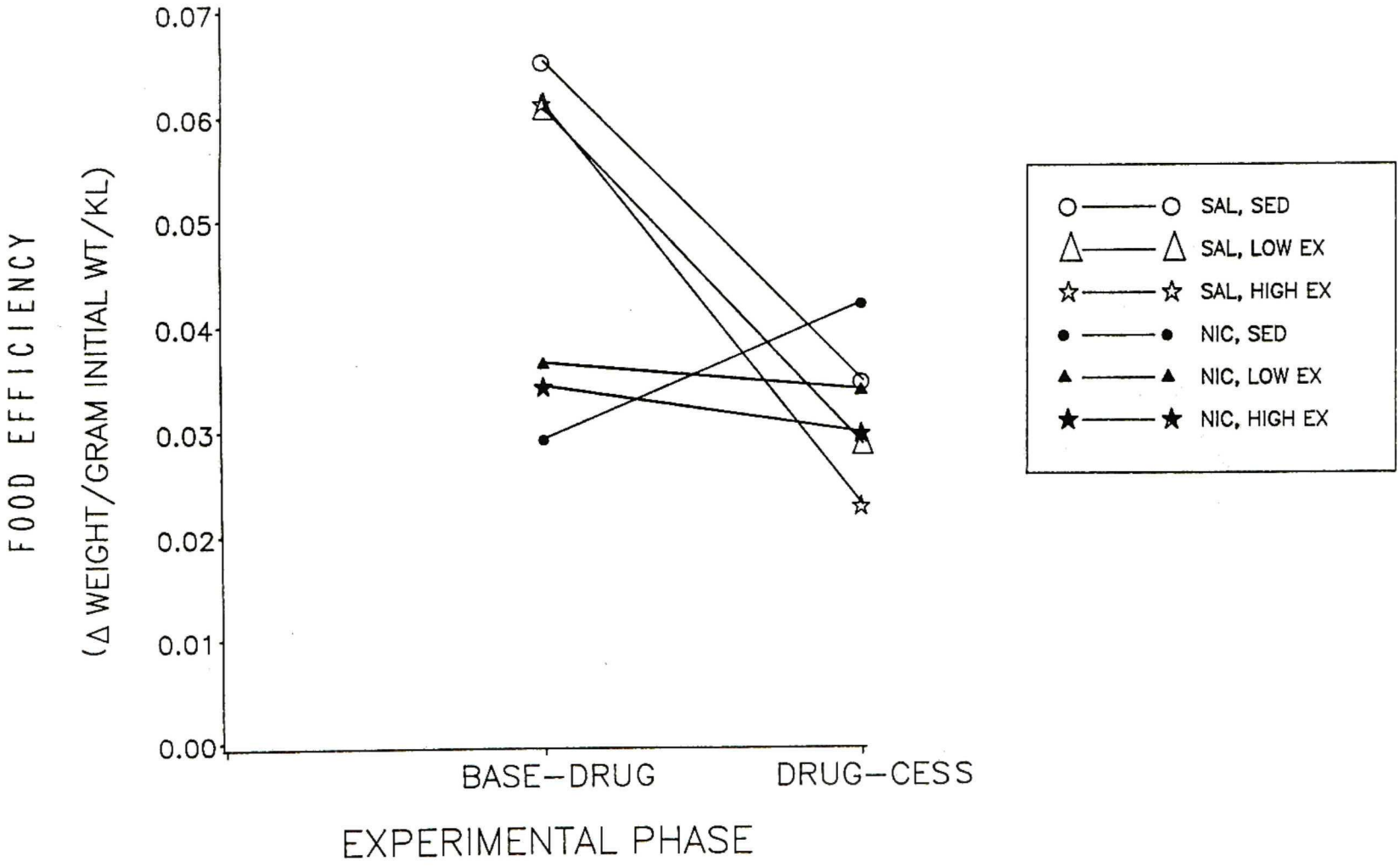


Figure 17
MEAN CESSATION WEIGHT CHANGE

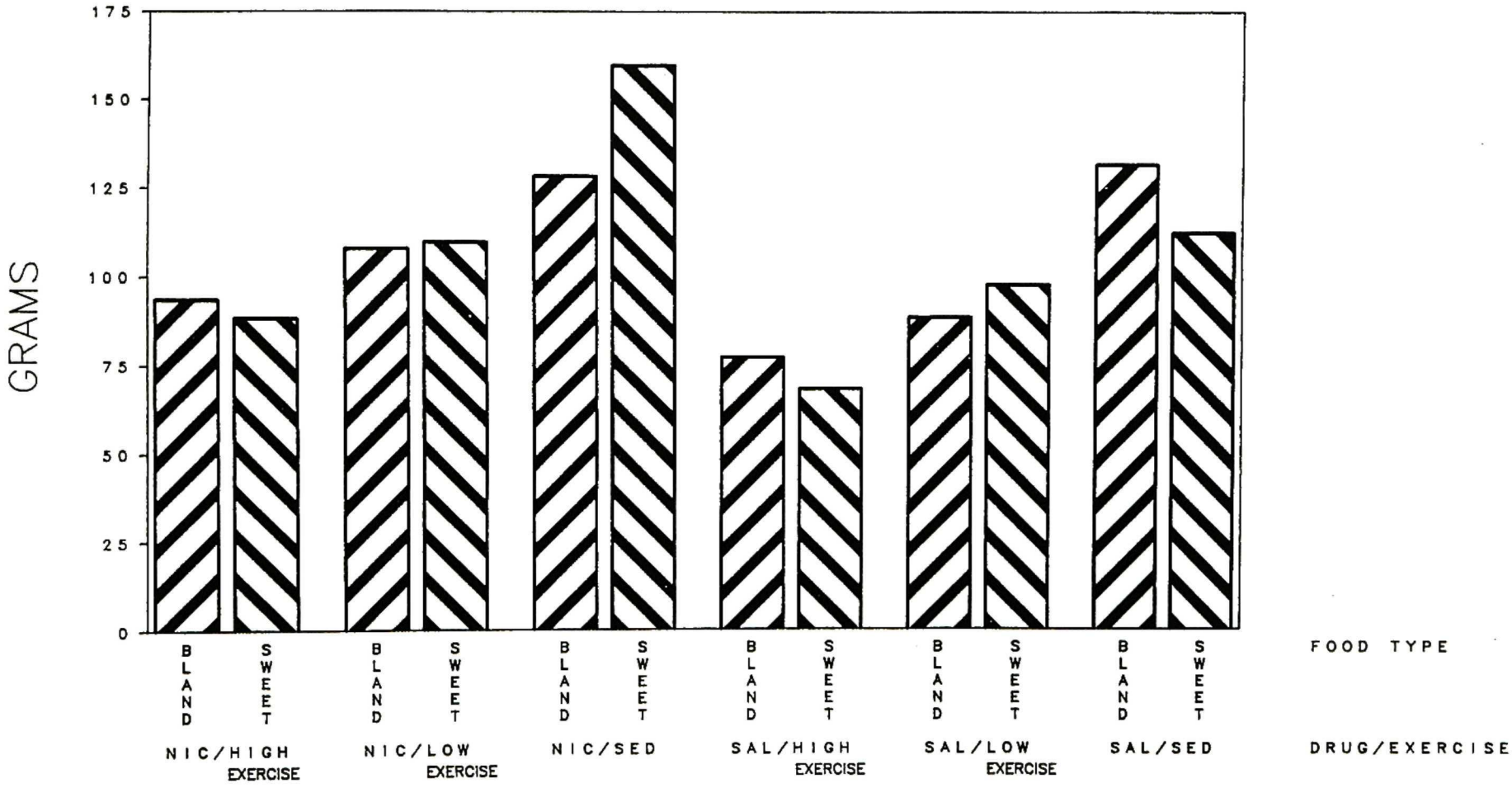


Figure 18
MEAN PLASMA INSULIN LEVELS

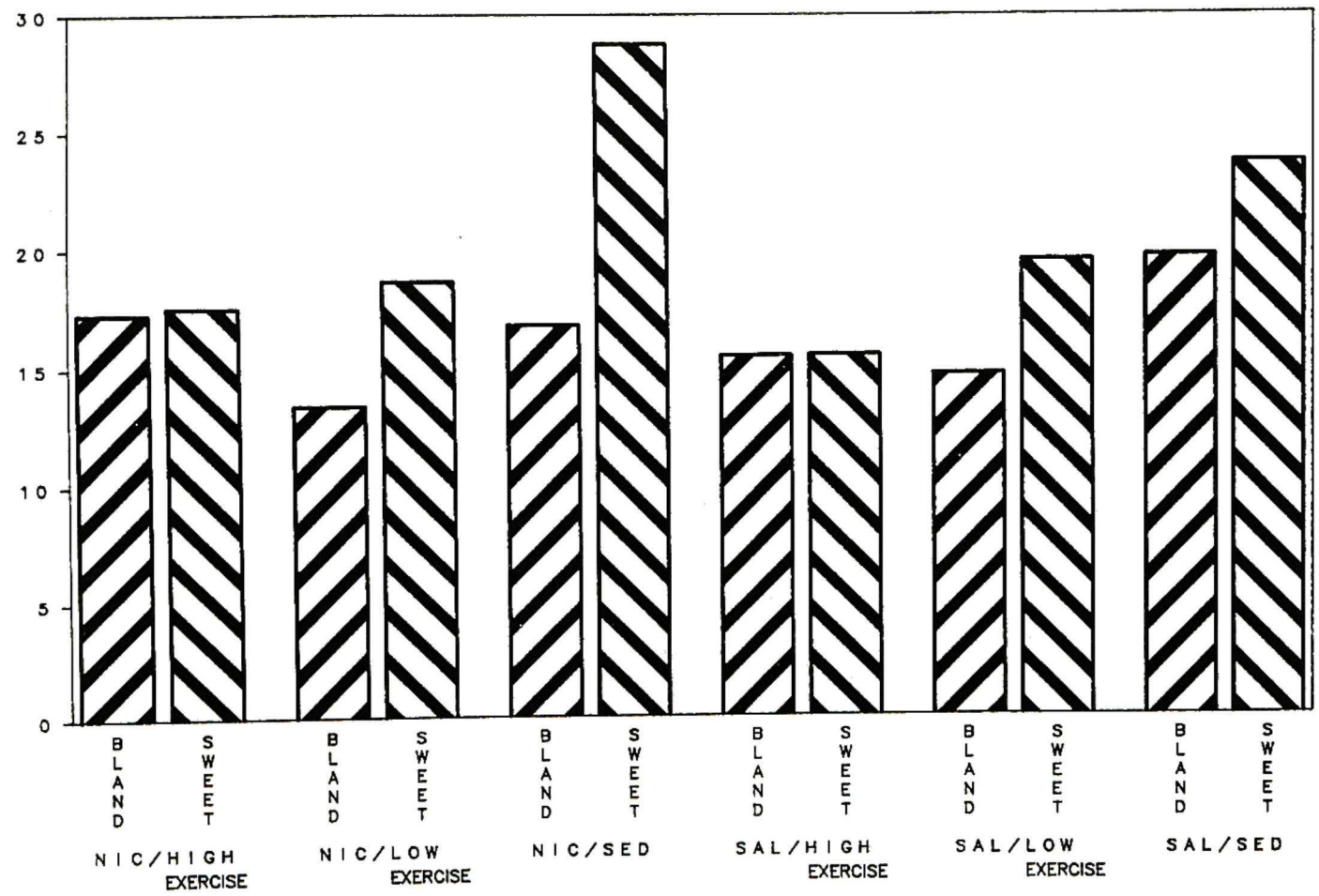


Figure 19
FAT INDEX

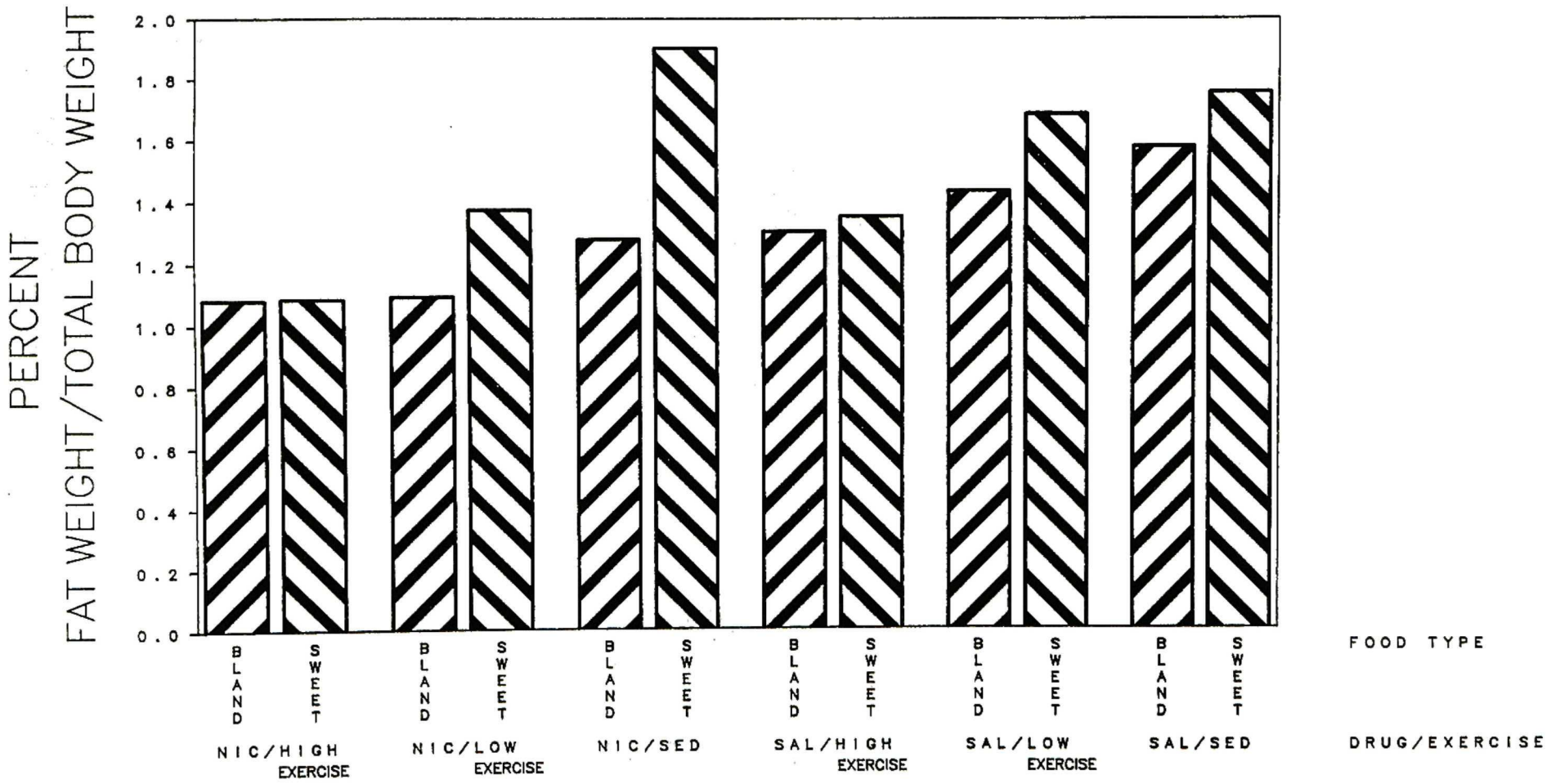
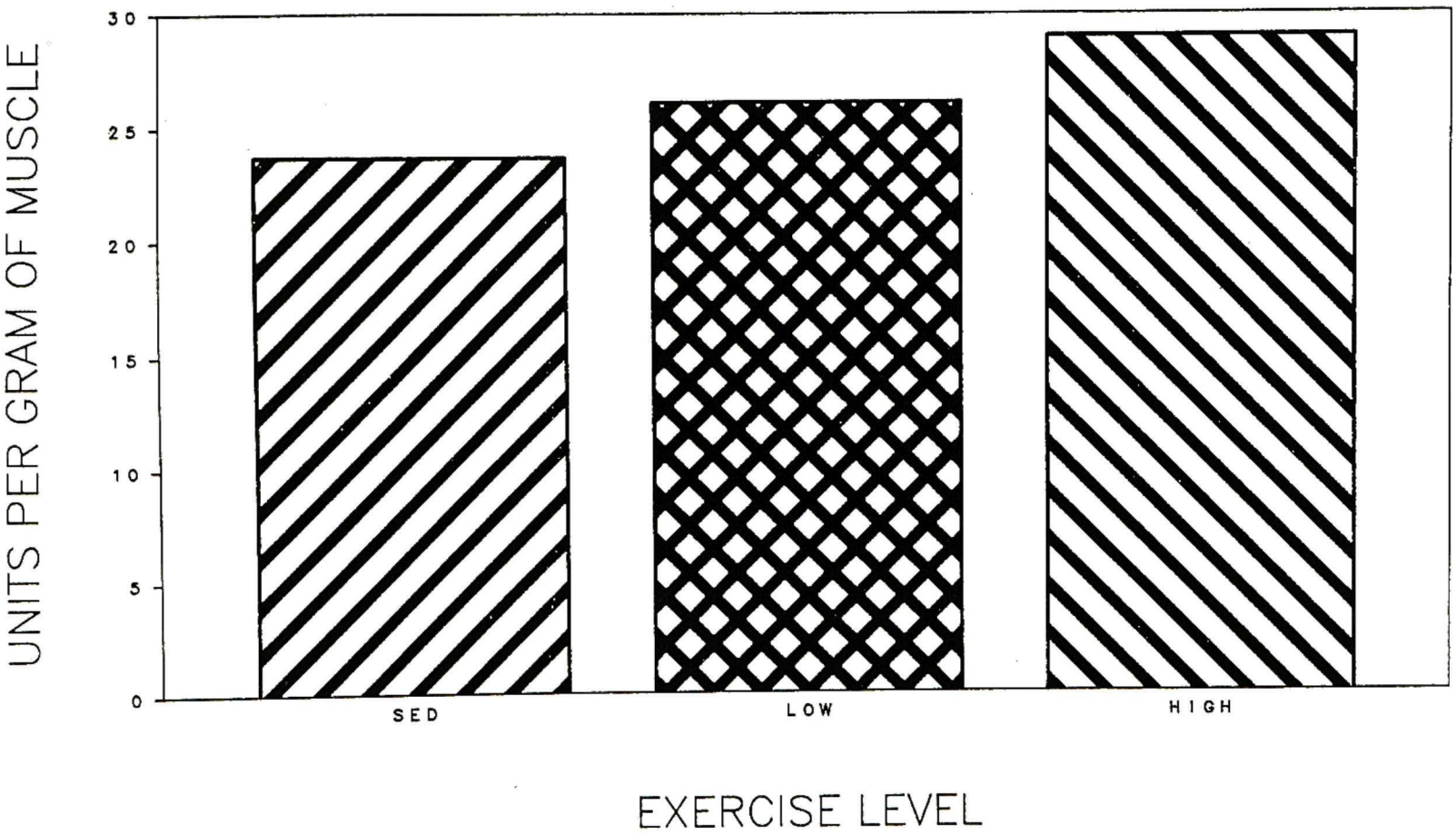


Figure 20
GASTROCNEMIUS CITRATE SYNTHASE



APPENDIX 1

Physical Training Schedule

Day of Training	Length of Swim Session Low Exercise Group	Length of Swim Session High Exercise Group
1	5.0 min.	10.0 min.
2-6	7.5 min.	15.0 min.
7	Rest	Rest
8	10.0 min.	20.0 min.
9	12.5 min.	25.0 min.
10-13	15.0 min.	30.0 min.
14	Rest	Rest
15	17.5 min.	35.0 min.
16	20.0 min.	40.0 min.
17-20	22.5 min.	45.0 min.
21	Rest	Rest
22	25.0 min.	50.0 min.
23	27.5 min.	55.0 min.
24-27	30.0 min.	60.0 min.
28	Rest	Rest
29-34	30.0 min.	60.0 min.

APPENDIX 2

Citrate Synthase Assay Procedure

This assay measures citrate synthase activity by linking the production of the sulfhydryl form of Coenzyme A (CoA-SH) from a reaction between Acetyl-CoA and oxaloacetate catalyzed by citrate synthase to the production of mercaptide ions in the presence of 5,5-dithiobis-(2-nitrobenzoate) (DTNB). This procedure is based on Srere's (1969) procedure and personal communication with Dr. Roland Savard (December, 1989).

Muscle Tissue Collection. Right gastrocnemius muscles were rapidly excised and immediately frozen in liquid nitrogen. They were then placed in small plastic jars and packed in dry ice for 1-2 hours and then stored in a freezer at -70 degrees Celsius until the assay was performed.

Enzyme Extraction. Approximately 200 mg of muscle from a horizontal medial slice of the gastrocnemius was weighed, immersed in 10 volumes of 175 mM KCl at pH 7.4 in a 14 ml test tube, and stored on ice until homogenization (less than 15 minutes). The test tube was then placed in a plastic beaker of ice water and the tissue was homogenized approximately 30 seconds using a polytron (Brinkmann Instruments). Care was taken to avoid frothing during homogenization by keeping the speed of the polytron as low as possible while still homogenizing the tissue. The homogenate was immediately frozen in a freezer at -70 degrees to preserve as much enzyme activity as possible. Homogenates were then thawed at room temperature and frozen two more times at -70 degrees Celsius to disrupt cell membranes, including those surrounding the mitochondria where citrate synthase is located. After the third

freeze, the homogenates were thawed at room temperature a third time, swirled by hand for ten seconds, and immediately centrifuged at 3000 rpm for 20 minutes at four degrees Celsius. One hundred microliters of supernatant was drawn from each sample and transferred to separate test tubes and placed on ice. Each 100 ul aliquot of supernatant was diluted with 300 ul of 100 mM TRIS buffer pH 8.4 (made fresh daily - see section on reagents), mixed by swirling and placed back on ice until assayed.

Reagents. All reagents were prepared fresh daily.

1. 100 mM TRIS buffer

6.055 g of TRIS base (MW = 121.1) was dissolved in 500 ml distilled water. 12N HCl was added to adjust the pH to 8.4 at room temperature (23 Celsius).

2. 1 mM DTNB (Sigma)

3.96 mg DTNB (MW = 396.4) was dissolved in 100mM TRIS buffer and brought to a 10 ml total volume.

3. 10 mM Oxaloacetate (Sigma)

1.321 mg cis oxaloacetic acid (MW = 132.1) was dissolved in 100 mM TRIS buffer and brought to a total volume of 1 ml.

4. 3 mM Acetyl CoA (Na salt) (Sigma)

3.1 mg Acetyl CoA (free acid MW = 809.6) was dissolved in distilled water and brought to a 1 ml total volume.

5. Citrate synthase standards (Sigma)

20 ul of citrate synthase crystalline suspension from pigeon breast muscle (85 units per mg protein; 2.4 mg protein/ml) were resuspended in 4.060 ml of 100 mM TRIS buffer, creating a solution containing 1 unit of citrate synthase per ml. The pigeon breast source for citrate synthase was used because it was the only skeletal muscle source available. Appropriate dilutions were made to prepare solutions of 0.25, 0.50, and 0.75 units per ml. All four concentrations were used to calculate the standard curve.

Procedure.

1. Zero spectrophotometer (Gilford 250 in this study) using the following blank solution (total volume is 1 ml) composed of: 0.70 ml TRIS buffer, 0.10 ml DTNB, 0.15 ml acetyl CoA, 0.05 ml oxaloacetic acid. A new blank solution was prepared every half hour.

2. Reagents were added to the cuvettes (4 ml square polystyrene for this study) in the following order: 0.69 ml TRIS buffer, 0.10 ml DTNB, 0.15 ml Acetyl CoA, 0.01 ml muscle solution (diluted supernatant). Any change in absorbance was followed for three minutes to measure possible acetyl-CoA deacylase activity. The citrate synthase reaction was then started by adding 0.05 ml oxaloacetic acid. Change in absorbance was recorded every 30 seconds for 3 minutes. The mean change in absorbance per minute was calculated for each duplicate of each sample. The mean change in absorbance per minute was then calculated for each duplicate pair.

The slope and intercept of the standard curve were calculated using linear regression and were used to calculate

citrate synthase activity for each sample. These values were then multiplied by 40 (the dilution factor) to obtain the citrate synthase activity per g of muscle tissue.

APPENDIX 3

Repeated Measures ANOVA
Body Weight

Multivariate Comparison Over Time

Time	F(2,76)	=	821.26	p < .0001
Time x Diet	F(2,76)	=	1.91	p < .16
Time x Drug	F(2,76)	=	104.49	p < .0001
Time x Diet x Drug	F(2,76)	=	3.73	p < .03
Time x Exercise	F(4,154)	=	11.49	p < .0001
Time x Diet x Ex	F(4,154)	=	1.16	p < .33
Time x Drug x Ex	F(4,154)	=	0.49	p < .74
Time x Diet x Drug x Ex	F(4,154)	=	1.53	p < .20

Repeated Measures ANOVA
Caloric Consumption

Multivariate Comparison Over Time

Time	F(2,76)	=	19.54	p < .0001
Time x Diet	F(2,76)	=	17.47	p < .0001
Time x Drug	F(2,76)	=	20.82	p < .0001
Time x Diet x Drug	F(2,76)	=	2.23	p < .12
Time x Exercise	F(4,154)	=	2.54	p < .05
Time x Diet x Ex	F(4,154)	=	1.67	p < .16
Time x Drug x Ex	F(4,154)	=	1.43	p < .23
Time x Diet x Drug x Ex	F(4,154)	=	0.54	p < .71

Repeated Measures ANOVA
Bland Food Consumption

Multivariate Comparison Over Time

Time	F(2,76)	=	7.50	p < .002
Time x Diet	F(2,76)	=	18.81	p < .0001
Time x Drug	F(2,76)	=	2.66	p < .08
Time x Diet x Drug	F(2,76)	=	5.38	p < .007
Time x Exercise	F(4,154)	=	2.45	p < .05
Time x Diet x Ex	F(4,154)	=	0.38	p < .83
Time x Drug x Ex	F(4,154)	=	0.22	p < .93
Time x Diet x Drug x Ex	F(4,154)	=	1.77	p < .14

Repeated Measures ANOVA
Sweet Food Consumption

Multivariate Comparison Over Time

Time	$F(2,39) =$	44.29	$p < .0001$
Time x Drug	$F(2,39) =$	9.54	$p < .0005$
Time x Exercise	$F(4,80) =$	1.72	$p < .16$
Time x Drug x Ex	$F(4,80) =$	0.94	$p < .45$

Repeated Measures ANOVA
Water Consumption

Multivariate Comparison Over Time

Time	F(2,76)	=	16.74	p < .0001
Time x Diet	F(2,76)	=	4.37	p < .02
Time x Drug	F(2,76)	=	12.98	p < .0001
Time x Diet x Drug	F(2,76)	=	0.83	p < .44
Time x Exercise	F(4,154)	=	1.32	p < .27
Time x Diet x Ex	F(4,154)	=	0.41	p < .80
Time x Drug x Ex	F(4,154)	=	0.54	p < .71
Time x Diet x Drug x Ex	F(4,154)	=	0.39	p < .82

Repeated Measures ANOVA
Food Efficiency

Multivariate Comparison Over Time

Time	F(1,77)	=	99.06	p < .0001
Time x Diet	F(1,77)	=	3.66	p < .06
Time x Drug	F(1,77)	=	122.94	p < .0001
Time x Diet x Drug	F(1,77)	=	5.51	p < .03
Time x Exercise	F(2,77)	=	5.51	p < .006
Time x Diet x Ex	F(2,77)	=	0.83	p < .45
Time x Drug x Ex	F(2,77)	=	1.63	p < .21
Time x Diet x Drug x Ex	F(2,77)	=	0.12	p < .89

Repeated Measures ANOVA
Body Weight: Contrasts

Baseline – Drug Cessation				
Diet	F(1,77)	=	0.43	p < .52
Drug	F(1,77)	=	12.89	p < .0006
Diet x Drug	F(1,77)	=	0.01	p < .93
Exercise	F(2,77)	=	14.03	p < .0001
Diet x Exercise	F(2,77)	=	0.65	p < .53
Drug x Exercise	F(2,77)	=	0.02	p < .98
Diet x Drug x Exercise	F(2,77)	=	3.01	p < .06

Drug Administration – Drug Cessation				
Diet	F(1,77)	=	0.06	p < .81
Drug	F(1,77)	=	9.64	p < .003
Diet x Drug	F(1,77)	=	1.72	p < .20
Exercise	F(2,77)	=	26.56	p < .0001
Diet x Exercise	F(2,77)	=	0.53	p < .60
Drug x Exercise	F(2,77)	=	0.10	p < .91
Diet x Drug x Exercise	F(2,77)	=	2.25	p < .12

Repeated Measures ANOVA
Caloric Consumption: Contrasts

Baseline – Drug Cessation				
Diet	F(1,77)	=	19.33	p < .0001
Drug	F(1,77)	=	0.00	p < .99
Diet x Drug	F(1,77)	=	3.51	p < .07
Exercise	F(2,77)	=	4.88	p < .02
Diet x Exercise	F(2,77)	=	1.28	p < .29
Drug x Exercise	F(2,77)	=	2.15	p < .13
Diet x Drug x Exercise	F(2,77)	=	0.08	p < .93

Drug Administration – Drug Cessation				
Diet	F(1,77)	=	2.97	p < .09
Drug	F(1,77)	=	11.17	p < .002
Diet x Drug	F(1,77)	=	4.51	p < .04
Exercise	F(2,77)	=	4.56	p < .02
Diet x Exercise	F(2,77)	=	0.07	p < .94
Drug x Exercise	F(2,77)	=	1.21	p < .31
Diet x Drug x Exercise	F(2,77)	=	0.15	p < .87

Repeated Measures ANOVA
Bland Food Consumption: Contrasts

Baseline	- Drug Cessation			
Diet	F(1,77)	=	1.19	p < .28
Drug	F(1,77)	=	5.13	p < .03
Diet x Drug	F(1,77)	=	0.23	p < .64
Exercise	F(2,77)	=	2.93	p < .06
Diet x Exercise	F(2,77)	=	0.17	p < .85
Drug x Exercise	F(2,77)	=	0.26	p < .78
Diet x Drug x Exercise	F(2,77)	=	3.29	p < .05

Drug Administration	- Drug Cessation			
Diet	F(1,77)	=	33.04	p < .0001
Drug	F(1,77)	=	0.57	p < .46
Diet x Drug	F(1,77)	=	6.41	p < .02
Exercise	F(2,77)	=	4.05	p < .03
Diet x Exercise	F(2,77)	=	0.53	p < .60
Drug x Exercise	F(2,77)	=	0.10	p < .91
Diet x Drug x Exercise	F(2,77)	=	0.73	p < .49

Repeated Measures ANOVA
Sweet Food Consumption: Contrasts

Baseline	–	Drug Cessation	
Drug	F(1,40) =	3.31	p < .08
Exercise	F(2,40) =	2.49	p < .10
Drug x Exercise	F(2,40) =	1.94	p < .40

Drug Administration	–	Drug Cessation	
Drug	F(1,40) =	16.09	p < .0004
Exercise	F(2,40) =	0.63	p < .54
Drug x Exercise	F(2,40) =	0.96	p < .40

Repeated Measures ANOVA
Water Consumption: Contrasts

Baseline - Drug Cessation				
Diet	F(1,77)	=	2.88	p < .10
Drug	F(1,77)	=	2.45	p < .13
Diet x Drug	F(1,77)	=	1.55	p < .22
Exercise	F(2,77)	=	2.06	p < .14
Diet x Exercise	F(2,77)	=	0.72	p < .49
Drug x Exercise	F(2,77)	=	0.52	p < .60
Diet x Drug x Exercise	F(2,77)	=	0.30	p < .74

Drug Administration - Drug Cessation				
Diet	F(1,77)	=	8.85	p < .004
Drug	F(1,77)	=	9.75	p < .003
Diet x Drug	F(1,77)	=	0.16	p < .69
Exercise	F(2,77)	=	1.95	p < .15
Diet x Exercise	F(2,77)	=	0.28	p < .76
Drug x Exercise	F(2,77)	=	0.49	p < .62
Diet x Drug x Exercise	F(2,77)	=	0.42	p < .67

Repeated Measures ANOVA
Food Efficiency: Univariate ANOVAs

Food Efficiency 1			
Diet	F(1,77)	= 0.95	p < .34
Drug	F(1,77)	= 88.50	p < .0001
Diet x Drug	F(1,77)	= 5.38	p < .03
Exercise	F(2,77)	= 0.02	p < .98
Diet x Exercise	F(2,77)	= 0.75	p < .48
Drug x Exercise	F(2,77)	= 1.45	p < .25
Diet x Drug x Exercise	F(2,77)	= 0.28	p < .76

Food Efficiency 2			
Diet	F(1,77)	= 5.47	p < .03
Drug	F(1,77)	= 24.73	p < .0001
Diet x Drug	F(1,77)	= 0.09	p < .77
Exercise	F(2,77)	= 29.13	p < .0001
Diet x Exercise	F(2,77)	= 2.01	p < .15
Drug x Exercise	F(2,77)	= 0.12	p < .89
Diet x Drug x Exercise	F(2,77)	= 0.23	p < .80

Repeated Measures Anova
Drug Main Effects & Interactions

Body Weight				
Drug	$F(1,77)$	=	157.80	$p < .0001$
Diet x Drug	$F(1,77)$	=	3.58	$p < .07$
Daily Caloric Consumption				
Drug	$F(1,77)$	=	40.57	$p < .0001$
Diet x Drug	$F(1,77)$	=	0.39	$p < .54$
Sweet Food Consumption				
Drug	$F(1,40)$	=	13.98	$p < .0007$
Bland Food Consumption				
Drug	$F(1,77)$	=	2.12	$p < .15$
Diet x Drug	$F(1,77)$	=	9.85	$p < .003$
Food Efficiency				
Drug	$F(1,77)$	=	122.94	$p < .0001$
Diet x Drug	$F(1,77)$	=	5.51	$p < .03$
Water Consumption				
Drug	$F(1,77)$	=	19.20	$p < .0001$
Diet x Drug	$F(1,77)$	=	1.40	$p < .24$

MANOVA Analyses

1 Cessation Weight Gain

Diet	F(5,73)	=	12.07	p < .0001
Drug	F(5,73)	=	8.75	p < .0001
Diet x Drug	F(5,73)	=	0.89	p < .50
Exercise	F(10,148)	=	5.44	p < .0001
Diet x Exercise	F(10,148)	=	0.56	p < .85
Drug x Exercise	F(10,148)	=	0.58	p < .83
Diet x Drug x Exercise	F(10,148)	=	0.55	p < .86

2 Sweet Food Consumption

Drug	F(6,35)	=	4.91	p < .001
Exercise	F(12,72)	=	3.18	p < .002
Drug x Exercise	F(12,72)	=	0.47	p < .9300

3 Food Efficiency

Diet	F(3,75)	=	3.11	p < .04
Drug	F(3,75)	=	12.81	p < .0001
Diet x Drug	F(3,75)	=	0.24	p < .88
Exercise	F(6,152)	=	7.86	p < .0001
Diet x Exercise	F(6,152)	=	0.93	p < .47
Drug x Exercise	F(6,152)	=	0.31	p < .94
Diet x Drug x Exercise	F(6,152)	=	0.26	p < .96

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